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# CEREAL CHEMISTRY

Published by the American Association of Cereal Chemists

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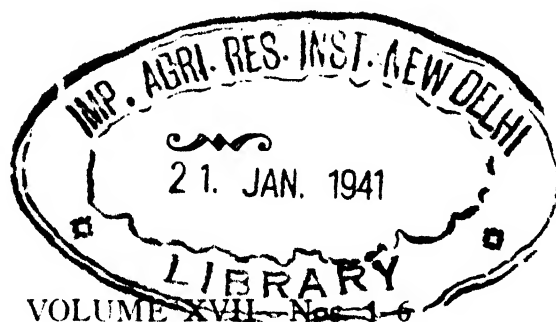
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VOLUME XVII - Nos 1-6

JANUARY-NOVEMBER, 1940



Lancaster, Pennsylvania

1940



# CEREAL CHEMISTRY

VOL. XVII

JANUARY, 1940

No. 1

## THE EFFECT OF MILK<sup>1</sup> ON THE BROMATE REQUIREMENTS OF FLOURS<sup>2</sup>

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(Received for publication July 29, 1939)

Although the use of milk in domestic bread making doubtlessly dates from antiquity, its application in commercial baking is of comparatively recent origin. It is difficult to say what purposes first led to its use but it is reasonable to assume that the increase in nutritive property of the bread was an important consideration. Certainly that has been given as an important reason for its use, and various investigations have been conducted to prove that milk improves the nutritional value of bread made with it as one of the components of the formula. Sherman *et al.* (1921), Morison and Amidon (1923), and Fairbanks (1938) have demonstrated that inclusion of milk in the baking formula materially improved the value of bread as a single diet. This is a conclusion that could be reached by *a priori* reasoning if it were assumed that the digestibility of the milk components was not impaired during the processes of fermentation and baking.

Early in the use of milk in commercial bread making it was observed that different preparations varied in their effects on the bread. A number of investigations were conducted to determine the causes of these variations and to discover the conditions necessary for producing milk preparations of uniform, satisfactory baking quality, and good nutritive value. Greenbank *et al.* (1927) and Grewe and Holm (1928) found that the preheating of skim milk before drying was an important factor influencing the quality of the dried product. The latter authors concluded that temperatures between 73°C. and 93°C. gave satisfactory results. They also observed that the preheating increased the viscosity of the water suspension of the powdered milk.

<sup>1</sup> The term milk is used to indicate dry-milk solids, the product obtained by removal of water from skim milk.

<sup>2</sup> Contribution No. 64 from the Department of Milling Industry, condensed from a thesis submitted to the graduate faculty, Kansas State College, in partial fulfillment of the requirements for the degree of Master of Science.

<sup>3</sup> Research fellow, American Dry Milk Institute, and Professor of Milling Industry, Kansas State College, respectively.

Johnson and Ward (1936) showed that although the viscosity of non-sweetened milk was increased by preheating and could also be increased by other methods, viscosity is not necessarily a good indication of the baking quality of the preparations. Fairbanks and Mitchell (1935) observed that the proteins of milk powder made from skim milk that had been preheated at temperatures high enough to produce scorching were reduced in biological value by as much as 20%, and were deficient in lysine. As normal milk and unscorched dry-milk solids are not deficient in this amino acid, it seemed evident that it is susceptible to damage by too high temperatures during the preheating process.

Skovholt and Bailey (1932b) found that the baking quality of a series of dried milks could be ranked fairly well by the plasticity curves obtained on a farinograph and with salt as an ingredient of the doughs. The amount of decrease in dough plasticity with extended mixing was found to give a significant correlation with baking quality of various dried-milk preparations.

The effect of dry-milk solids on the physical properties of doughs has been studied in attempts to obtain a better understanding of the manner in which this material functions. St. John and Bailey (1929b) found that extensibility of the dough was not materially affected by the inclusion of 10% or less of dry-milk solids. Skovholt and Bailey (1932b) observed that inclusion of dry-milk solids in wheat flour doughs increased the time required to reach the maximum plasticity as indicated by the farinograph. This was corroborated by Bohn and Bailey (1937), who pointed out also that the stress readings of a dough after mixing are markedly decreased by the inclusion of dry-milk-solids-not-fat.

A number of other investigations have revealed some interesting results accruing from the inclusion of dry-milk solids in the baking formula. Amidon (1926) found a general optimum of baking qualities at a dry-milk-solids level of approximately 7%. Grewe (1928) reported that the volume, grain, break, shred, and color were improved by the addition of dry-milk solids to doughs. She also reported that the tendency for the break to "run wild" was reduced and that the range of fermentation time over which good bread could be obtained was increased by the addition of dry-milk solids. St. John and Bailey (1929a) showed that total production of  $\text{CO}_2$  in yeast-leavened doughs was increased when dry-milk solids was superimposed on the control formula. The rate of increase in volume and total displacement of doughs was practically the same with or without milk in the formula. The buffer action of dry-milk solids was appreciable as shown by the initial hydrogen-ion concentration of the freshly mixed doughs and

the relative rate of change in pH of the control and milk-containing doughs. Skovholt and Bailey (1931, 1937) found that dry-milk solids suppresses the saccharogenic activity of flour-water suspensions and that reduction in diastatic activity is the result of reduced hydrogen-ion concentration effected by the dry-milk solids. They stated that gas production from fermenting doughs was accelerated by dry-milk solids if sufficient sugar was available.

Working (1928) observed that when dry-milk solids were used in no-time doughs an improvement was obtained with addition of both a phosphatide and an oxidizing agent. Skovholt and Bailey (1932a) presented data which indicated that either malt or Arkady used in conjunction with dry-milk solids gave complementary effects.

In this brief review it can be observed that much of the earlier work has been directed toward learning how to prepare milk suited to bread making, ascertaining the best dosages to use, and attempting to measure the effects of milk on the physical properties of doughs. As a result of these investigations, satisfactory milk preparations are now available to the commercial bakers and increasing quantities are being used by the industry. Other improving agents are also being used in large quantities, particularly with the hard red winter wheat flours. Most important of these are the chlorine-type bleaching agents and certain "flour improvers," the majority of which contain potassium bromate as the active "oxidizing" component. As milk and bromate may each improve flours, it is a matter of importance to know whether their actions are supplementary, complementary, or independent. The work reported in this paper was directed toward the problem of the effect of dry-milk solids on the bromate requirements of various types of flours, with particular emphasis on the hard red winter class.

### Materials and Methods

The flours used in this investigation are described in Table I. They were chosen as representative of the various types used in commercial bread production in different parts of the country, as well as flours which are used for the production of cake, pastries, and alimentary pastes. They consisted of commercially milled and experimentally milled flours, both bleached and unbleached. Two of the flours (Nos. 10 and 11, an unbleached Tenmarq and an unbleached Chiefkan) were extracted with ethyl ether for further investigation. This extraction was performed in an enlarged extractor of the Soxhlet type for a period of at least 24 hours. After extraction the flours were exposed to the atmosphere for a time sufficient to remove all traces of ether.

Dry-milk solids is the product resulting from the removal of water from liquid skim milk and contains less than 5% moisture and less

TABLE I

FLOURS USED, THEIR PROTEIN CONTENTS, BLEACHING TREATMENTS, AND THE METHODS BY WHICH THEY WERE MILLED

Sam- ple No.	Wheat variety or class from which flour was milled	Protein, 13.5% moisture basis	Bleaching treatment	Method of milling
1	Low-protein composite from Turkey, Tenmarq, and Blackhull	% 9.9	Unbleached	Experimental
2	High-protein composite from Turkey, Tenmarq, and Blackhull	14.4	Unbleached	Experimental
3	Hard red winter (Kan.)	11.5	Bleached	Commercial
4	Hard red spring (Minn.)	14.7	Unbleached	Commercial
5	Hard red spring (Can.)	13.3	Unbleached	Commercial
6	Soft red winter (Mo.)	9.3	Bleached	Commercial
7	Soft red winter (Mo.)	10.3	Unbleached	Commercial
8	Pacific short patent (Oreg.)	6.8	Bleached	Commercial
9	Durum (N. D.)	11.1	Unbleached	Experimental
10	Tenmarq (Kan.)	9.8	Unbleached	Commercial scale, experimental mill
11	Chiefkan (Kan.)	11.5	Unbleached	Commercial scale, experimental mill

than 1½% fat. The dry-milk solids used in this investigation was manufactured by the spray process. A sample of this milk was compared with a sample of known baking quality and gave comparable results in a baking test. The dry-milk solids was incorporated in the dough by mixing the dry ingredients thoroughly with the flour before the addition of any of the other baking ingredients.

The baking procedure used was a modification of the standard method approved by the American Association of Cereal Chemists. The formula involved the use of the ingredients listed below and the quantities indicated. Percentages of ingredients are based on flour as 100 per cent. Formula: Flour 100%; water as required; yeast 2%; sugar 6%; salt 1.75%; shortening 3%; bromate and dry-milk solids in variable quantities. Baking absorption was determined by a method developed by Finney.<sup>4</sup> This was increased 1% for each 1% of dry-milk solids except where 8% of dry-milk solids was used, in which case the absorption increase was the same as that for the 6% dry-milk-solids doughs. This procedure was followed because previous experience indicated that the doughs became too sticky to handle properly if this increase was exceeded. Doughs were mixed until they attained an optimum consistency as determined by visual observation. Finney

<sup>4</sup> Paper read at the tri-section meeting of the American Association of Cereal Chemists, Manhattan, Kansas, 1939.

and Barmore<sup>5</sup> have shown that this procedure is preferable to mixing for a definite period of time for all samples. The doughs were mixed from 200 g. of flour, divided into two equal parts, fermented, and proofed at 86°F. The doughs were fermented and proofed according to the time schedule from the standard method of the American Association of Cereal Chemists. Punching was done with a National pup sheeting roll and molding of the loaves by a Thompson laboratory scale molder. The loaves were baked in tall narrow pans at 232°C. in a Despatch oven with rotating hearth. Loaf volume was measured immediately upon removal of the loaves from the oven. A National pup-loaf measuring device was used to determine the volume. The figures for loaf volume given in the tables are averages of two loaves measured in this manner. The loaves were cut the day following baking for scoring of the interior characteristics and for obtaining a photographic record of the interior grain structure.

### Discussion of the Data

Preliminary investigations showed that the amount of potassium bromate necessary to produce optimum loaves of good texture was greater when milk was included in the formula than when it was absent. The amount of bromate varied with the protein content of the flours, those of high protein requiring more than those of low protein content.

In order to investigate this further, two winter-wheat-flour composites were prepared from the supplies of experimentally milled flour available. The composites were made from approximately equal amounts of Turkey, Tenmarq, and Blackhull, using samples of the protein-variety series described by Larmour, Working, and Ofelt (1939). The lower-protein sample had 9.9% protein and the higher, 14.4%. While these samples do not represent the extremes of the range in protein content in the 1938 crop, they provided fairly representative samples of about the lowest and highest protein that might be expected to occur in commercial bread flours. They were baked in two series, with and without dry-milk solids (6%), each series having varying amounts of potassium bromate in the formulas. The baking data are given in Table II (samples 1 and 2) and are shown graphically in Figure 1.

The low-protein flour without milk showed a distinct maximum with 0.001% bromate. With greater amounts of bromate the loaf volume decreased and texture became poorer. When milk was used the optimum occurred with 0.002% bromate, and with increasing amounts up to 0.004% there was no diminution in volume or texture

<sup>5</sup> Paper read at Annual Meeting of American Association of Cereal Chemists at Kansas City, 1939.



TABLE II

BAKING DATA SHOWING THE INTERRELATIONSHIP BETWEEN DRY-MILK SOLIDS (DMS) AND  $\text{KBrO}_3$  WITH VARIOUS FLOURS

Sample No.	Treatment	Dosage of $\text{KBrO}_3$ (Mg./100 g. flour)											
		0		1		2		3		4		5	
		Loaf vol.	Texture	Loaf vol.	Texture	Loaf vol.	Texture	Loaf vol.	Texture	Loaf vol.	Texture	Loaf vol.	Texture
1	Milk-free 6% DMS	610 738	68 75	655 770	68 75	625 805	63 73	603 808	50 78	600 805	55 77	555 770	40 71
2	Milk-free 6% DMS	708 755	76 69	883 865	93 88	905 913	85 90	830 925	78 89	745 998	75 89	760 1003	75 89
3	Milk-free 6% DMS	715 770	95 96	735 835	90 98	693 825	83 98	690 860	79 89	673 822	73 93	663 803	71 93
4	Milk-free 6% DMS	768 817	85 93	888 960	90 95	770 993	70 80	695 978	63 80	690 895	60 75		
5	Milk-free 6% DMS	688 823	83 93	710 888	79 92	635 888	73 92	570 845	64 84	568 813	60 68	538 733	54 65
6	Milk-free 6% DMS	600 720	68 88	565 738	55 85	550 700	55 83	533 708	50 80	530 700	50 75	515 675	45 75
7	Milk-free 6% DMS	618 768	65 79	625 790	71 77	622 690	49 83	595 818	50 78	600 780	52 78	578 762	47 70
8	Milk-free 6% DMS	490 580	50 53	455 560	45 49	463 —	43 —	460 —	43 —	440 —	40 —	438 —	40 —
9	Milk-free 6% DMS	485 513	50 60	463 513	43 48	443 513	35 45	435 493	25 33	— —	— —	— —	— —

score. There was thus a "plateau" between 0.002% and 0.004% bromate over which constant values for volume and texture were obtained.

The high-protein flour behaved somewhat differently. Without milk it gave an optimum at 0.002% bromate, although this was only slightly higher than at 0.001% bromate. The increase in volume due to bromate was great, and the decrease due to overdosage was also great. With the 6% milk, the volumes were the same as for "without milk" until the 0.002% dosage was reached. Thereafter the doughs with milk continued increasing in volume instead of decreasing as the checks did. There was no significant difference between the 0.004% and 0.005% dosages; evidently the maximum had been reached with 0.004% bromate.

With both low and high-protein flours the optimum dosage of bromate was twice as great in the milk doughs as in the check doughs. It should be noted too that at optimum dosage of bromate the low-

protein sample showed greater effects due to milk than did the high-protein sample, the increases over the best volumes obtained in the checks being 153 and 98 cc. respectively.

The most important observation in this experiment was that in the presence of 6% dry-milk solids it is possible to use one dosage of bro-

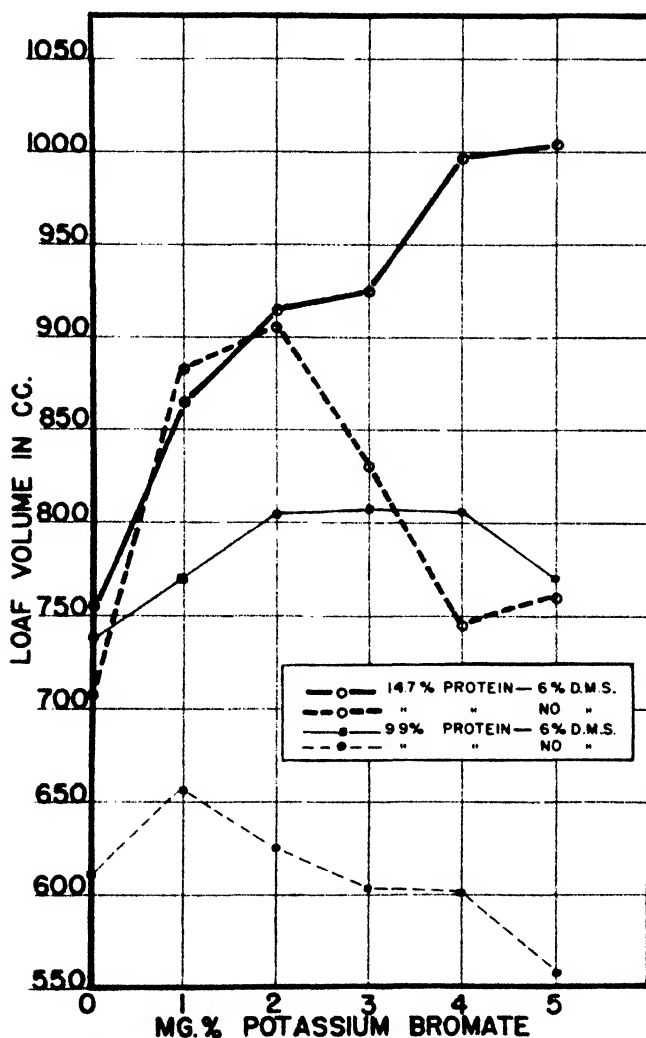


Fig. 1. Baking data showing the interrelationship between dry-milk solids and  $\text{KBrO}_3$  on two flours, one from a low-protein composite wheat (No. 1, Table I) and the other from a high-protein composite wheat (No. 2, Table I).

mate that will give nearly optimum baking results with both low- and high-protein winter-wheat flours. In Figure 1 it can be seen that 0.004% bromate brought the high-protein flour to what may have been the beginning of its "plateau" and carried the low-protein flour to the end of its "plateau" or optimum range. This is a curious condition, which if applicable to flours in general would prove of great value in

both experimental and commercial baking. In experimental baking it would mean that a sufficiently high dosage of bromate could be used to ensure optimum development of the high-protein samples without

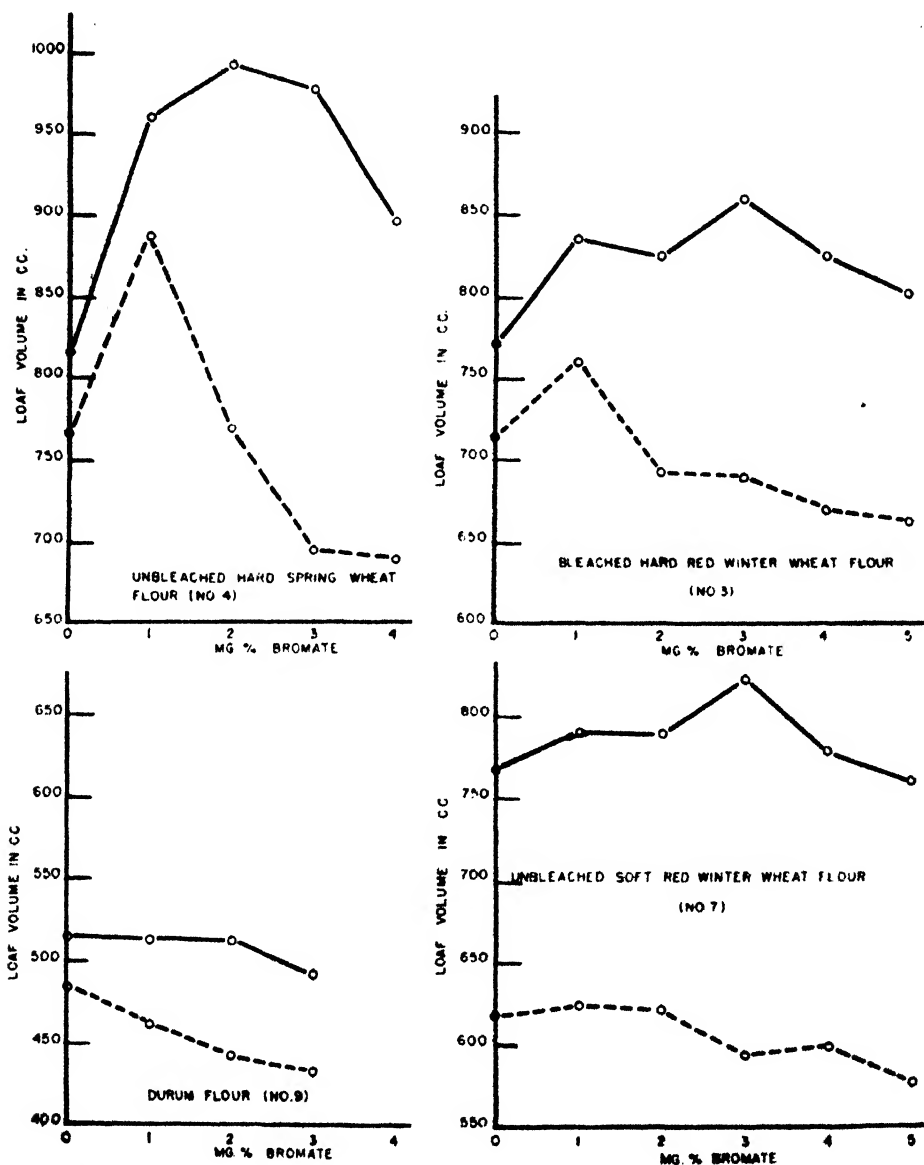


Fig. 2. Baking data showing the interrelationship between dry-milk solids and  $\text{KBrO}_3$  on three commercial flours (No. 3, 4, and 7, Table I) and an experimentally milled durum flour (No. 9). Broken line represents milk-free doughs; solid line represents doughs made with 6% dry milk solids.

the risk of damaging those of lower bromate requirements. In commercial practice it would mean that there would be much less danger of "overoxidizing" flours that had been brought close to their optimum condition in the process of manufacture.

In order to learn whether or not these observations, obtained with experimentally milled hard winter wheat flours, were applicable to commercially-milled flours, and particularly to different classes of flour, a series composed of various types was tested in the manner described above. Description of these samples is given in Table I. The baking data are presented in Table II. (The data obtained with several of the more interesting flours are shown graphically in Figure 2.)

All these flours except the very low-protein Pacific short patent flour gave results somewhat comparable to those obtained with samples 1 and 2. It is true that not all of them would tolerate 0.004% bromate even with 6% milk present, but they showed the common characteristic of having a range of tolerance toward bromate in the presence of dry-milk solids much broader than in the plain doughs.

TABLE III

BAKING DATA SHOWING THE INTERRELATIONSHIP BETWEEN DRY-MILK SOLIDS (DMS) AND  $\text{KBrO}_3$  ON NORMAL, UNBLEACHED CHIEFKAN AND TENMARQ FLOURS AND ON THE SAME FLOURS FOLLOWING EXTRACTION WITH ETHYL ETHER

Flour	Treatment	Dosages of $\text{KBrO}_3$ (Mg./100 g. flour)											
		0		1		2		3		4		5	
		Loaf vol.	Texture	Loaf vol.	Texture	Loaf vol.	Texture	Loaf vol.	Texture	Loaf vol.	Texture	Loaf vol.	Texture
Chiefkan (No. 11)	Milk-free 6% DMS	535	39	688	81	680	81	650	78	638	78	—	—
		572	20	717	75	750	100	792	100	730	95	710	80
Extracted Chiefkan	Milk-free 6% DMS	503	45	595	75	618	73	598	63	538	63	—	—
		555	59	658	75	695	75	680	75	680	73	—	—
Tenmarq (No. 10)	Milk-free 6% DMS	600	60	593	60	558	55	550	50	543	50	545	50
		730	65	750	70	752	75	720	80	690	80	680	75
Extracted Tenmarq	Milk-free 6% DMS	495	50	490	50	480	50	475	50	473	48	480	48
		615	70	605	68	605	70	550 <sup>1</sup>	70	613	73	625	70

<sup>1</sup> This sample received improper treatment in the molding equipment.

Sample No. 3, Table II, a commercial, bleached hard winter wheat flour of 11.5% protein content, showed an abrupt optimum with 0.001% bromate without milk. With milk there was an optimum at 0.003% bromate and it seems likely that the range of bromate tolerance was 0.001% to 0.004%. Even with 0.005% bromate and 6% milk there was not a serious diminution of volume or texture. This sample showed characteristics similar to No. 1, the low-protein experimentally milled flour.

A commercial, unbleached hard red spring wheat flour milled in Minnesota, of 14.7% protein (Sample No. 4, Table II), gave a very

sharp maximum value for loaf volume with the milk-free formulas, characterized by a sharp rise at 0.001% bromate dosage, followed by a decrease equal to the rise at 0.002% dosage. Further decrease occurred with increasing amounts of bromate. When the milk was included in the formula the optimum range was at least 0.002% to 0.003% bromate (probably 0.001% to 0.003%). It is generally recognized that the hard spring wheat flours require somewhat less "oxidation" than the hard winter flours, and the shorter range of bromate tolerance noted in this flour is doubtlessly a reflection of this characteristic.

Sample No. 5 was a commercial, unbleached flour obtained from a large Canadian mill. It was said to be a first clear flour, and as such could not be regarded as typical of Canadian spring wheat flours. It proved to be a very interesting sample, however, and the data have been included here because they represent an example of low positive response to bromate (either with or without milk), high susceptibility to overbromating, and a very marked positive response to the presence of dry-milk solids in the baking formula. The milk increased the bromate tolerance to 2 mg., but this was the lowest range observed except in the case of sample No. 10, which will be discussed later.

Several samples of soft winter wheat flour were also studied. First to be tested was a commercial bleached soft red winter wheat flour milled in Eastern Missouri and said to be typical of the flour produced from that class of wheat. Later another sample, unbleached, was obtained from the same mill. The two samples were of 9.3% and 10.3% protein respectively. It is evident that sample No. 6 had been treated to the limit in milling, because it did not show any tolerance for bromate in the absence of milk; the best volume and texture were obtained with the unbromated dough. With milk there was a slight but scarcely significant increase in volume with 0.001% bromate. This was accompanied by a decrease in texture score. However, there was no great decrease in volume in this series until a dosage of 0.005% bromate had been reached.

The unbleached soft red winter wheat sample, No. 7, gave very little positive response to bromate either with or without milk. Without milk there was no effect on volume from 0.001% and 0.002% bromate, but in the latter case the texture fell off 22 points. Higher dosages gave somewhat lower volumes but no further reduction in texture score. With milk there was a small increase in volume due to bromate but the whole range of volume change was only 56 cc. Neither was there a great deal of variation in texture in the series. It is worth noting, however, that with 0.002% bromate the texture of the samples treated with milk was high, while that of the milk-free sample

had dropped to the low value of 49, which indicates very poor texture indeed. Thus while there is not much evidence of effect of milk on the volumes, it does appear to maintain the texture.

The Pacific short patent flour, sample No. 8, was very low in protein and was a typical cake flour. It was included in this series merely as a matter of interest. It was not possible to handle the doughs made with 6% milk and bromate in excess of 0.001%. The data obtained indicate that this flour would not tolerate bromate in even 0.001% dosage. But in the plain doughs there was little decrease in volume. The flour was not sensitive and was too weak to be regarded as a bread flour.

Sample No. 9 was experimentally milled from durum wheat obtained from North Dakota. It was reputed to be a "typical" sample, although the protein content was too low to be representative of the crop. The response to bromate was negative, though not great, in the plain doughs. Milk reduced the negative volume response almost to zero and improved the texture considerably.

The soft-wheat and durum-wheat flours used in this study showed very little volume response to the action of bromate either with or without milk. There was a notable effect of milk in these samples on the textures of the bread. With the plain formulas bromate damaged the texture when used in the higher dosages but did not materially diminish the volume; milk reduced this damaging effect greatly.

Among the hard winter wheats there are many varieties which show quite a large range of characteristics. The varieties Tenmarq and Chiefkan probably represent the extremes. Tenmarq is said to possess some of the characteristics of its Marquis parent. It probably requires less bleaching than most winter-wheat varieties; it has a relatively long mixing time; it exhibits the long wheat-meal-fermentation time of the spring wheats; and there is considerable evidence that it needs less bromate than the average hard winter wheat. Chiefkan, on the other hand, has very short mixing time, usually gives short wheat-meal-fermentation time, and requires heavy dosages of bromate. The differences in their baking behavior can be seen in the data in Table III and in the graphs in Figure 3.

With milk-free doughs Tenmarq gave evidence of not requiring any bromate, while Chiefkan showed a great increase in both volume and texture with 0.001% bromate. With 6% milk Tenmarq gave a slight increase in volume and considerable improvement in texture on addition of bromate up to 0.002%; greater amounts of bromate decreased the volume but not the texture. The Chiefkan sample showed progressive increase in volume and a most astonishing improvement in texture with bromate up to 0.003%.

Perhaps the most outstanding difference of the Chiefkan doughs was in connection with their "feel," a property real enough to the baker but one almost impossible to describe clearly. These doughs lacked "resiliency," they "tended to flatten out on standing;" they were "soft and rather inelastic." These properties were particularly

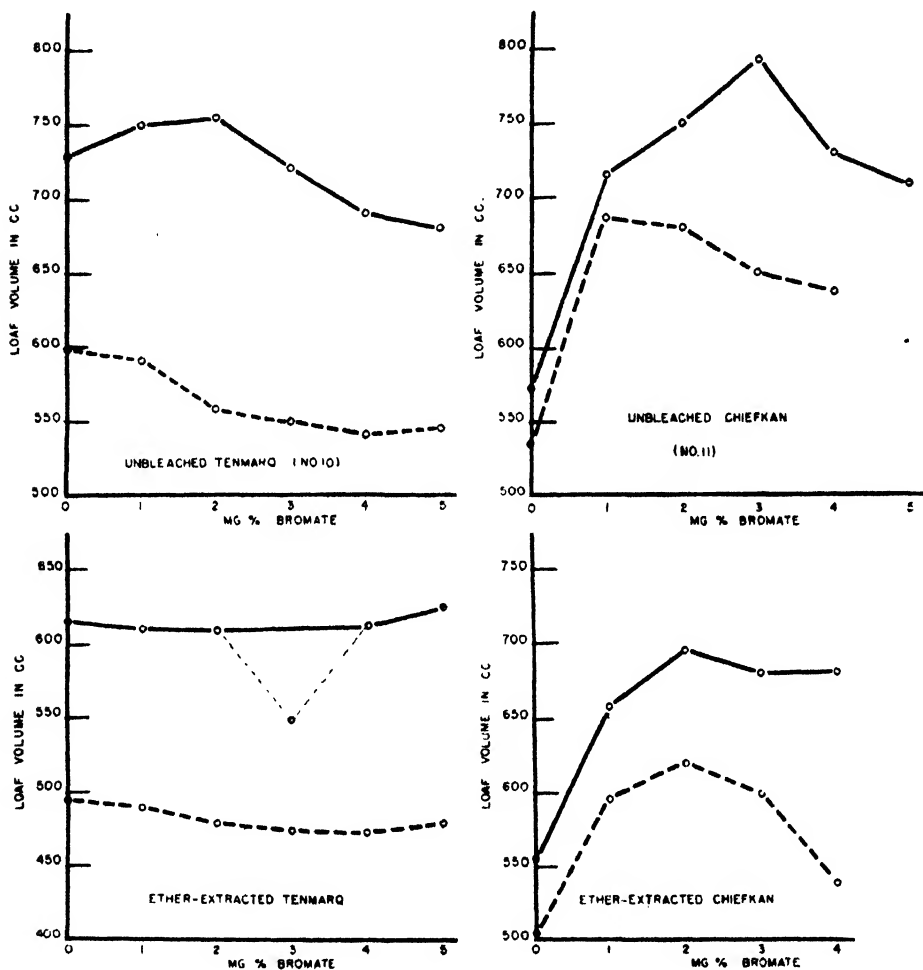


Fig. 3. Baking data showing the inter-relationship between dry-milk solids and  $\text{KBrO}_3$  on Tenmarq (No. 10, Table I) and Chiefkan (No. 11) flours and also on the same flours following extraction with ethyl ether. Broken line represents milk-free doughs; solid line represents doughs made with 6% dry milk solids.

noticeable in the unbromated doughs but they persisted to some extent in the bromated doughs also. With both milk and bromate the doughs were similar to standard hard winter flour doughs. Some of these peculiar dough characteristics were like those of dough made from flour that had deteriorated through overaging.

Kozmin (1935) and Barton-Wright (1938) have shown that unsaturated fatty acids may be responsible for profound changes in the

colloidal nature of doughs and have suggested that they may be the cause of the damage occurring in overaging. Removal of these fatty acids by ether extraction restores, in part or in whole, the original properties of the flour. Having in mind the possibility that the differences between Chiefkan and Tenmarq flours might be due to the presence of free fatty acids or other similarly acting substances in varying amounts, 2500-g. samples of each flour were extracted with ether in a large size continuous extractor constructed by E. B. Working, and subsequently baked with various amounts of bromate. The data are given in Table III and curves showing the relation of loaf volume to bromate dosage in Figure 3.

There was no evidence of decreased differentiation of these flours as a result of ether extraction. The Chiefkan flour still responded notably to bromate, while the Tenmarq flour gave practically no response. The loaf volume level was lowered approximately 50 cc. for Chiefkan and 100 cc. for Tenmarq. The loaf volume and texture response to milk were very little changed at all bromate levels. It thus seems evident that the differences in dough characteristics of these two flours were not attributable to differences in naturally occurring amounts of any ether-soluble substance.

There was next investigated the possibility that these two flours differed in the amount or activity of protease. If the theory advanced by Jørgenson (1935, 1935a, 1936, 1939) and supported by Balls and Hale (1936, 1938) and Flohil (1936), that bromate owes its effect to inhibition of protease activity in flour, were correct, the differences in bromate response of these two flours might very well be accounted for on this basis. The Chiefkan ought to have the higher protease content, or activity, because it produced exceedingly poor bread, without bromate, and showed a remarkable improvement upon its addition, whereas the Tenmarq produced good bread without bromate and only slightly better when it was added. It has been shown above that the differences in bread characteristics were reduced greatly by the use of the optimum amount of bromate for each flour, namely 0.003% for Chiefkan and 0.002% for Tenmarq. If this were due to different protease contents or activities, a contrary effect should be obtained by addition of protease activators; their differences ought to be accentuated.

With these considerations in mind, the two flours were baked with two dosages of cysteine monohydrochloride, 1 mg. and 5 mg. per 100 g. of flour. The data in Table IV show that with milk-free doughs there was no change in loaf volume due to cysteine in either flour. With 6% milk there was a small decrease in volume, which may have been



TABLE IV

BAKING DATA ON UNBLEACHED TENMARQ AND CHIEFKAN FLOURS SHOWING THE COMPARATIVE EFFECTS OF VARYING CONCENTRATIONS OF CYSTEINE MONO-HYDROCHLORIDE ON MILK-FREE DOUGHS AND DOUGHS CONTAINING 6% DRY-MILK SOLIDS (DMS)

Flour	Treatment per 100 g. flour	Milk-free doughs		6% DMS	
		Vol- ume	Score of crumb	Vol- ume	Score of crumb
		cc.		cc.	
Tenmarq	Control (No bromate)	600	60	730	65
	1 mg. cysteine mono- hydrochloride	608	78	695	85
	5 mg. cysteine mono- hydrochloride	598	78	678	88
Chiefkan	Control (No bromate)	535	39	572	20
	1 mg. cysteine mono- hydrochloride	535	46	538	33
	5 mg. cysteine mono- hydrochloride	538	46	543	33

attributable to secular variability, as the checks were not baked at the same time as the samples with cysteine. There was no significant difference between loaves having 1 and 5 mg. of the cysteine. In all cases the texture scores of bread made with cysteine in the formula were better than those of the checks. In the baking results, the only effect that could be attributed to cysteine was an improvement in texture. There was certainly no differential effect on the two flours.

An important observation made in the course of this experiment was that cysteine caused a very definite decrease in mixing time of all doughs in which it was included. This effect was somewhat greater with the higher dosage. Chiefkan, naturally a short mixing flour, became almost too sticky to handle after mixing one minute in the Swanson-Working mixer. Tenmarq, which normally required 5 minutes of mixing, was reduced to about 2.5 minutes. The effect of cysteine seems to occur almost instantaneously. This was also shown in the recording dough-mixer curves by a very sharp rise to the maximum and a rapid drop and diminution of width of the curve. The speed with which cysteine affects the dough appeared to be much too rapid to be attributed to enzyme action. It had the characteristics of a colloidal effect.

The results of this experiment provide no evidence of increased differentiation attributable to a protease activator. One can conclude that protease content or activity was not the factor responsible for the great differences in bromate response of these two flours.

### Specific Effects of Milk

The response of these different flours to milk is difficult to assess properly because the bromate requirements are different for milk-free doughs and those containing milk. Direct comparisons at any one level of bromate dosage are bound to be misleading. Perhaps the most admissible comparisons would be on the non-bromated doughs, but even in that case the natural differences in bromate requirement of the flours would tend to give incorrect information. With certain flours such as the soft red winters which have little response to bromate, the approximate effect of milk might be estimated at almost any level of bromate without very great error. This would not be true of flours such as the hard red springs or hard winters because the milk-free doughs may be actually overbromated, resulting in decreased volume and texture, while those with milk are still short of their optimum condition.

The changes in loaf volume attributable to the presence of 6% dry-milk solids, at all bromate levels studied, are given in Table V.

TABLE V

IMPROVEMENT IN LOAF VOLUME RESULTING FROM THE INCLUSION OF 6% DRY-MILK SOLIDS IN THE FORMULA

Sample No.	Differences in loaf volumes between milk-free doughs and doughs containing 6% dry-milk solids at various dosages of bromate (Mg./100 g. flour)						Diff. in vol.— optimum milk doughs and milk-free doughs
	0	1	2	3	4	5	
1	128	115	180	205	205	215	153
2	47	18	8	95	253	243	98
3	55	100	132	170	149	140	125
4	49	72	223	273	205	—	105
5	153	178	253	265	245	195	178
6	120	173	150	175	170	160	138
7	150	165	168	213	180	184	193
9	28	50	70	58	—	28	28
10	130	157	194	170	147	152	152
11	37	29	70	142	98	104	104

In the last column of the table the differences between the best loaves of the check series and the best loaves of the series containing 6% dry-milk solids are given. For example with the commercial hard red spring flour No. 4, the value 105 cc. was obtained by subtracting the loaf volume 887 cc., obtained with 0.001% bromate, from the loaf-volume figure 992 cc., obtained with 0.003% bromate and 6% dry-milk solids. These values represent the extent to which 6% dry-milk solids improves the loaf volume beyond the optimum obtainable with bromate alone.

Flours that have slight response to bromate tend in general to exhibit the greater response to milk. With Nos. 1 and 2, which possessed the same inherent quality but were different in protein content, the low-protein sample No. 1 gave small response to bromate and relatively large response to milk (based on the milk-free, non-bromated check samples, column 0). The high-protein sample No. 2 showed the converse effect, large response to bromate and small response to milk. Comparisons of Nos. 10 and 11, and Nos. 4 and 7, show similar results.

When one compares these values with those given in the last column of Table V, it is seen that they agree in a general way. One might predict the nature of the differences to be found in "net response" to milk in these three pairs of flours, but the relative responses obtained from the non-bromated doughs would be badly out of line with the "net responses."

In the foregoing comparisons the greater effect of milk occurred with the low-protein samples, and one might be inclined to associate low protein, low bromate response, and large "net milk" response. The hard red spring unbleached clear flour, No. 5, provides an exception because it was relatively high in protein content, low in bromate response, and high in "net response" to milk. That would, however, still leave the possibility of an association between low-bromate and high-milk responses were it not for the durum sample, No. 9, which had small response to both bromate and milk. Despite these exceptions, it seems probable that there may be an inverse relationship between bromate and milk responses in patent or straight bread flours.

### Summary

A series of flours, including hard red spring, hard red winter, soft red winter, white, and durum wheat flours was studied in respect to the effects of potassium bromate and of dry-milk solids in the baking formulas. In general the inclusion of 6% dry-milk solids creates a tolerance toward bromate which tends to prevent damage to loaf volume and to grain and texture when large dosages of this reagent are used. Even when the effect is small for loaf volume it remains marked for grain and texture. This buffering effect toward bromate has important commercial significance because it provides a safeguard against the possibility of damaging flours that have already been brought close to their optimum "oxidation" condition by bleaching or by the addition of other oxidizing agents.

Examination of two of the flours which had been thoroughly extracted with ether indicated that this buffering effect was not associated with the ether-soluble components of the flours.

Treatment of two flours that responded quite differently to both bromate and milk, with cysteine-hydrochloride, a protease activator, resulted in no increased differentiation of the flours. This was interpreted to mean that the protease content of the flours was not responsible for the differences noted.

Dry-milk solids, together with the appropriate amounts of potassium bromate, produced increases in loaf volumes and improvements in texture beyond what could be obtained with optimum amounts of bromate alone. The extent of this response appeared to be inversely related to bromate response in most cases. Exceptions were noted in the cases of the durum and the unbleached hard red spring clear flours.

In certain flours greater improvements were obtained with milk alone than with the optimum dosage of bromate. This was particularly applicable to the low-strength flours. In all cases, however, milk gave some improvement in both loaf volume and texture over what could be obtained with bromate alone.

The variations in magnitude of response to milk are important commercially and deserve much more investigation.

#### Literature Cited

- Amidon, G. W.  
1926 The effect of dry skim milk on the properties of bread. *Baking Tech.* **5**: 270-274.
- Balls, A. K., and Hale, W. S.  
1936 Proteolytic enzymes of flour. *Cereal Chem.* **13**: 54-60.  
1938 The preparation and properties of wheat proteinase. *Cereal Chem.* **15**: 622-627.
- Barton-Wright, E. C.  
1938 Observations of the nature of the lipides of wheat flour, germ and bran. *Cereal Chem.* **15**: 723-738.
- Bohn, I. J., and Bailey, C. H.  
1937 Effect of fermentation, certain dough ingredients, and proteases upon the physical properties of flour doughs. *Cereal Chem.* **14**: 335-348.
- Fairbanks, B. W.  
1938 Improving the nutritive value of bread by the addition of dry milk solids. *Cereal Chem.* **15**: 169-179.
- and Mitchell, H. H.  
1935 Nutritive value of skim milk powders with special reference to the sensitivity of the milk proteins to heat. *J. Agr. Res.* **51**: 1107-1121.
- Flohil, J. T.  
1936 The effect of chemical flour improvers on proteolytic action in relation to the gas-retaining capacity of fermenting doughs. *Cereal Chem.* **13**: 675-689.
- Greenbank, G. R., Steinbarger, M. C., Deysher, E. F., and Holm, G. E.  
1927 Effect of heat treatment of skim milk upon the baking qualities of the evaporated and dried products. *J. Dairy Sci.* **10**: 335-342.
- Grewe, Emily  
1928 Effect of dry skim milk on baking quality of various flours. *Cereal Chem.* **5**: 242-255.
- and Holm, G.  
1928 Effect of variation in the method of manufacture on the baking quality of dry skim milk. *Cereal Chem.* **5**: 461-69.
- Johnson, A. H., and Ward, F. C.  
1936 The relation between the viscosity of non-sweetened condensed skim milk and baking quality. *Cereal Chem.* **13**: 550-559.

- Jørgenson, Holger  
 1935 Ein Beitrag zur Beleuchtung der hemmenden Wirkung von Oxidationsmitteln auf proteolytische Enzymtätigkeit: Über die Natur der Einwirkung von Kaliumbromat und analogen Stoffen auf die Backfähigkeit des Weizenmehles, I. *Biochem. Z.* **280**: 1.  
 1935a Über die Natur der Einwirkung von Kaliumbromat und analogen Stoffen auf die Backfähigkeit des Weizenmehles, II. *Biochem. Z.* **283**: 134.  
 1936 On the existence of powerful but latent proteolytic enzymes in wheat flour. *Cereal Chem.* **13**: 346-355.  
 1939 Further investigation into the nature of the action of bromates and ascorbic acid on the baking strength of wheat flour. *Cereal Chem.* **16**: 51-59.
- Kozmin, Natalie P.  
 1935 The aging of wheat flour and the nature of this process. *Cereal Chem.* **12**: 165-171.
- Larmour, R. K., Working, E. B., and Ofelt, C. W.  
 1939 Quality tests on hard red winter wheats. *Cereal Chem.* **16**: 733-752.
- Morison, C. B., and Amidon, George W.  
 1923 Milk in bread baking. *Baking Tech.* **2**: 210-214.
- St. John, J. L., and Bailey, C. H.  
 1929a Effect of dry skim milk on the fermentation and hydrogen-ion concentration of doughs. *Cereal Chem.* **6**: 51-59.  
 1929b Effect of dry skim milk upon the water absorption of doughs and the plasticity of flour suspensions. *Cereal Chem.* **6**: 140-150.
- Sherman, H. C., Rouse, M. E., Allen, B., and Woods, E.  
 1921 Growth and reproduction upon simplified food supply, I. *J. Biol. Chem.* **46**: 503-519.
- Skovholt, O., and Bailey, C. H.  
 1931 Relation of quality in dry skim milk to baking strength. *Cereal Chem.* **8**: 374-380.  
 1932a Dry skim milk and new crop flours. *Northwestern Miller and Am. Baker* **9**: 18.  
 1932b The effect of temperature and of the inclusion of dry skim milk upon the properties of doughs as measured with the farinograph. *Cereal Chem.* **9**: 523-530.  
 1937 The effect of milk solids on fermentation reactions. *Cereal Chem.* **14**: 108-119.
- Working, E. B.  
 1928 The action of phosphatides in bread dough. *Cereal Chem.* **5**: 223-233.

## QUALITY TESTS ON SOFT RED WINTER WHEATS OF KANSAS<sup>1</sup>

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(Received for publication July 21, 1939)

Soft red winter wheats represent a large percentage of the total wheat production of the United States, and in Europe they are the predominant type. They were used for making bread flours many centuries before the hard types, as we know them today, were introduced. Only within comparatively recent times have the hard wheats come to be regarded by many bakers as *necessary* in the manufacture of bread flours. Even today a large proportion of the bread flour of the world is made almost entirely from soft wheats.

<sup>1</sup> Contribution No. 63 from the Department of Milling Industry.

When the great central plains area of North America was opened up to farming, it was discovered that the climate and soil of the western part of the region were particularly well suited to the growing of wheat of a hard, vitreous character. As this type of wheat became available in commercial quantities, it was found that when mixed with the softer types the bread-making properties of the blends were superior and gradually new standards of quality in flour were established on this continent and in Europe.

There is no sharp line of demarcation between the hard-wheat and the soft-wheat regions in the United States. The line of demarcation lies in the approximate vicinity of the 95th meridian, but hard wheats are produced east of, and soft wheats are grown west of, this division line. There is an overlapping zone in which neither type is grown exclusively. As a consequence, soft wheats may be grown under conditions tending to produce a protein content higher than usual, and hard wheats may be grown under conditions tending to produce much lower protein than is customary. It is in this overlapping zone that the greatest difficulty is experienced with these two types or classes of wheat. The Eastern third of Kansas is within this area, and thus the problem is one of importance to this state.

It has been generally accepted as proved that the soft red winter wheats as a class are not only different in milling properties from the hard red winter and the hard red spring wheats, but also inferior to them in bread-making quality. This fact has been shown by the extensive investigations of Thomas (1917), Shollenberger (1923), Shollenberger and Clark (1924), Coleman *et al.* (1930), and the less extensive studies made by Pelshenke (1933), Geddes (1937) and others. Some of the data obtained by Thomas (1917) and by Shollenberger (1923) are shown in graphical form in Figure 1, and indicate that at all protein levels the soft red winter wheat flours gave lower loaf volumes than either the hard spring or hard winter wheat flours. Compared to either of these classes of hard wheats, the soft wheats would have to be regarded as definitely inferior. The average values given by Shollenberger and Clark (1924) are much less convincing. They show, for instance, that the hard spring wheats averaged 13.6% protein and 2,142 cc. in loaf volume, while the corresponding values for soft red winter wheats were 11.3% and 2,001 cc., respectively. It can be seen that the soft red winters although 16.9% lower in protein were only 6.6% lower in loaf volume than the hard red spring wheats of this series, thus making it appear that the soft wheats were of better quality intrinsically than the other classes of wheat represented. There is a contradiction in conclusions from these data and those of Shollenberger's (1923) earlier publication.

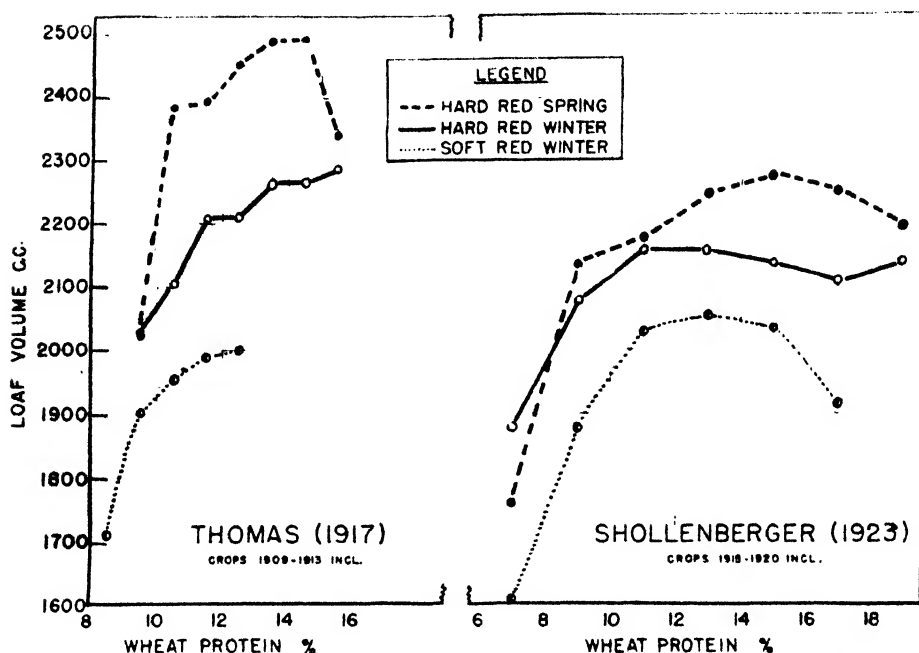


Fig. 1. Relation of loaf volume to wheat protein content for three classes of United States wheat.

A summary of the data of Coleman *et al.* (1930) is given in Table I. Here again, as in Shollenberger and Clark's (1924) data, it might be concluded that the loaf volumes of the soft red winter wheat flours were greater than they should have been for the protein content. Calculated from the hard red spring values, the volume at 10.26% protein would be expected to be 1,731 cc. instead of 2,152 cc., which it was. The soft red winter samples were, on the average, 0.2% lower in loaf volume although they were 19.7% lower in protein content. The only criticism of the baking performance of the soft wheats was

TABLE I

A SUMMARY OF THE DATA OBTAINED WITH EXPORT CARGO SAMPLES OF VARIOUS CLASSES OF UNITED STATES WHEAT  
From the data of Coleman *et al.* (1930)

Class	No. samples	Wheat protein	Loaf volume	Crumb		Break and shred	Loaf vol. per unit protein <sup>1</sup>
				Color	Grain		
Hard red spring	14	12.78	2156	87	91	Good	169
Hard red winter	34	10.91	2176	88	91	Fair	199
Soft red winter	40	10.26	2152	89	89	Poor	210
White wheat	30	10.94	2074	88	88	Fair	190

<sup>1</sup> Calculated from the data. Not in original data.

that the break and shred were poor as compared to "fair" for the hard winters and "good" for the hard spring wheat samples.

From Figure 1 it would seem that average values of samples covering a wide range of protein content ought to be accepted with some reservations. They may be vitiated by failure to get the loaf volume expected by high protein samples. It has been demonstrated by Larmour (1931), Geddes and Larmour (1933), Aitken and Geddes (1934), Geddes (1937), and others that with proper baking formulas the loaf volume of hard spring wheat flours is a linear function of the protein content. Larmour, Working, and Ofelt (1939) have shown that this applies also to the hard winter wheats. The earlier baking methods did not show this. On the contrary, the data of Thomas (1917) and Shollenberger (1923) indicate generally that the increase in loaf volume was not proportional to the increase in protein above a certain value. The higher protein samples were apparently no better and sometimes distinctly poorer than those of medium protein content. Consequently the average values obtained from such data would be influenced to different extents, depending on the relative proportion of samples falling in the high-protein classes. Therefore graphical representation such as shown in Figure 1 is more reliable than general averages.

Considering what is now known about experimental baking methods for both hard spring and hard winter wheats, it is doubtful that much reliance ought to be placed upon the earlier baking data which were obtained by the use of rather lean formulas, quite inadequate for use with high-protein samples. It was considered a matter of interest to see how far this applied to the soft red winter wheats.

Little has been published concerning the soft wheats of Kansas, because they represent a relatively small fraction of the total wheat production of the state. The Kansas State Board of Agriculture estimated that 8.3% of the total wheat acreage of Kansas was planted to soft winter wheats in 1937. The percentage production would be somewhat higher, because the soft wheats are grown principally in the eastern part of the state, where rainfall is relatively abundant and average yields are high.

The principal varieties grown are Kawvale, Clarkan, Harvest Queen, Michigan Wonder, Fulcaster, and Currell. The acreage sown to Kawvale exceeds that of all other soft varieties combined. In 1937 it amounted to 5.1% of the total wheat acreage in Kansas, or slightly more than 60% of the soft wheat acreage. Kawvale is a selection from Indiana Swamp, an old variety of limited distribution and is classed as a "semi-hard" wheat. Because Kawvale has good agronomic characteristics, it is grown over a fairly wide area and has caused



considerable difficulty from the standpoint of grading. Clarkan, Michigan Wonder, and Harvest Queen are typical soft wheats which meet the requirements of the soft-wheat millers quite satisfactorily. Of the three, Clarkan has the best agronomic characteristics and is the variety recommended for eastern Kansas.

### Materials and Methods Used

An excellent series of samples was obtained through the courtesy of A. L. Clapp of the Department of Agronomy of Kansas State College. The samples were grown in demonstration plots from seed supplied by the college; the harvesting and threshing were supervised by the college. The individual samples were first analyzed for protein, then combined into composites of various protein levels. The range of protein was not great, as the samples were grown in the area of higher rainfall, characteristic of the soft wheat regions.

The technique of testing was similar to that described in detail for Kansas hard winter wheats by Larmour, Working, and Ofelt (1939). For convenience the two baking formulas used are given in Table II.

TABLE II  
INGREDIENTS USED IN BAKING FORMULAS I AND II

Ingredients	Percentage based on flour	
	Formula I	Formula II
Yeast	2	2
Sugar	6	6
Shortening	3	3
Salt	1.50	1.75
Dry-milk solids	4	6
Potassium bromate	0.001	0.003 <sup>1</sup>
Water	As required	As required
Malt syrup (120° l.)	0.25	—
Ammonium phosphate	0.05	—

<sup>1</sup> Except with Kawvale and Turkey. With these 0.004% potassium bromate was used.

The doughs were mixed to optimum consistency, fermented three hours at 30° and proofed 55 minutes at the same temperature. They were baked 25 minutes at 232°C. The doughs were mixed in the Swanson-Working mixer, punched by means of National sheeting rolls, and molded in a Thompson laboratory mold.

Wheat-meal-fermentation time of the composite samples was determined by the method described by Swanson (1937), using 0.78 g. compressed yeast for each 15 g. meal.

Protein content, baking data by two formulas, wheat-meal-fermentation time, together with corresponding data for Turkey composites of the same protein range, are given in Table III.

TABLE III  
BAKING DATA AND DESCRIPTION OF SAMPLES

Variety	Flour protein	Formula I		Formula II		Wheat- meal-fer- mentation time
		Loaf vol.	Texture score	Loaf vol.	Texture score	
	<i>%</i>	<i>cc.</i>		<i>cc.</i>		<i>min.</i>
Clarkan	9.1	668	6.3	665	7.0	38
	10.4	665	5.9	705	7.0	41
	11.6	628	5.6	700	7.5	53
Harvest Queen	8.9	625	4.5	630	6.5	35
	10.1	668	4.9	700	7.0	36
	11.3	645	4.9	705	7.0	37
	11.7	655	5.6	715	7.0	39
Michigan Wonder	9.0	665	5.2	660	8.0	27
	9.6	712	5.2	715	8.0	34
	10.6	728	5.2	755	8.5	35
	12.0	725	4.9	765	8.5	39
Kawvale	10.1	700	7.7	723	9.5	58
	11.3	762	8.4	800	9.5	59
	13.0	752	8.1	863	9.5	63
	14.2	770	8.1	913	9.5	98
Turkey	8.2	708	8.3	658	8.0	23
	9.5	708	8.3	733	8.0	40
	10.1	752	9.0	743	8.5	57
	11.0	785	9.4	798	9.5	52
	11.7	822	9.1	843	9.5	49
	13.2	812	9.4	898	9.0	93

### Baking Data

Loaf volumes obtained by the two formulas for each of the varieties indicated in Table III are shown graphically in Figure 2. There was little difference in the loaf volumes obtained by the two formulas with the lowest protein samples of each variety. This indicates at least that the higher dosage of bromate in Formula II did not damage the lower-strength flours in the series. The beneficial effect of the combination of increased milk and increased bromate is shown in the higher-protein samples of each variety. While the relation of loaf volume to protein content of flour was not linear over the whole range of protein, it more closely approached linearity with baking Formula II. The increased milk and bromate benefited the high-protein flours more than those of low protein in each series.

Loaf volumes by Formula II are plotted against protein of flour in Figure 3. The resemblance of Kawvale to Turkey is evident. Michigan Wonder gave greater loaf volumes than either Clarkan or Harvest Queen. The two latter varieties were approximately equal and much lower than Turkey of corresponding protein content. There is no

doubt that these two soft wheat varieties were distinctly inferior to both Turkey and Kawvale in *inherent baking quality*. Michigan Wonder was somewhat higher in quality but not equal to Turkey.

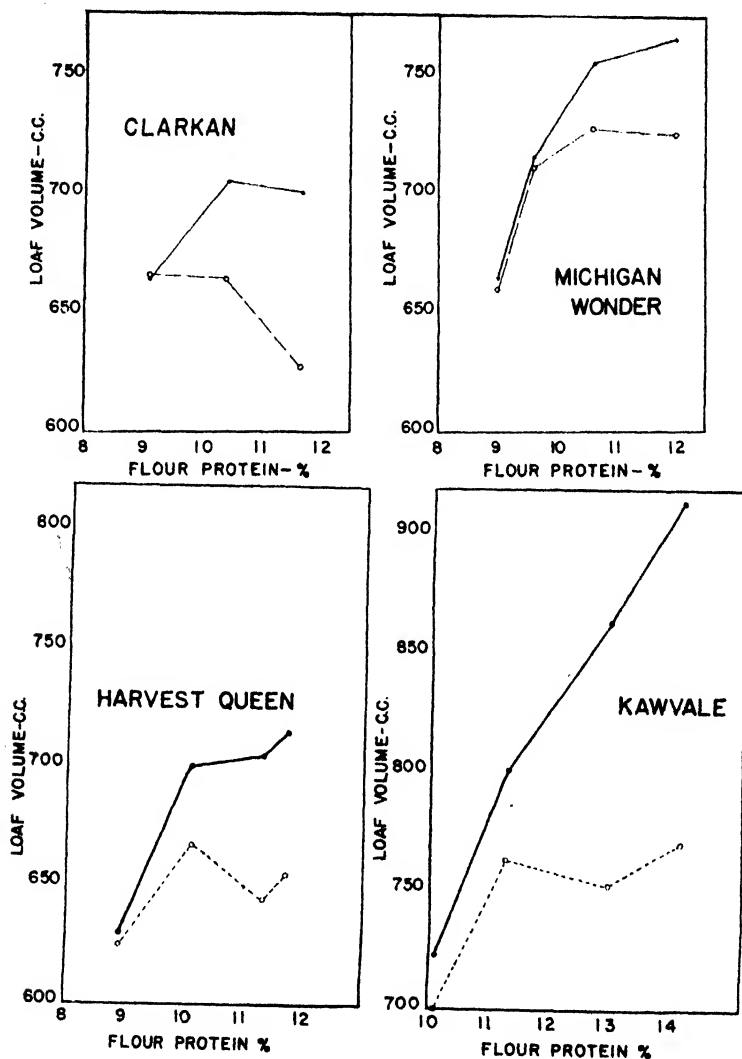


Fig. 2. Relation of loaf volume to flour protein content, for four varieties of Kansas wheat, crop of 1938. Broken graph represents Formula I, solid graph Formula II.

### Physical Characteristics of the Dough

All samples were mixed on the Swanson-Working recording micro-mixer and the curves so obtained are shown in Figure 4. The three soft wheats, Clarkan, Michigan Wonder and Harvest Queen, show some common characteristics, whereas the curves for Kawvale are distinctly different and resemble those obtained with Turkey. The soft-wheat

curves rise very abruptly to a maximum in about one minute and then fall off rather rapidly in both height and width. Kawvale on the contrary gives a relatively slow rise to the maximum, followed by a more gradual drop, which is not accompanied by the very rapid narrowing of the band shown by the other three varieties. These Kawvale curves indicate that the dough characteristics are closely related

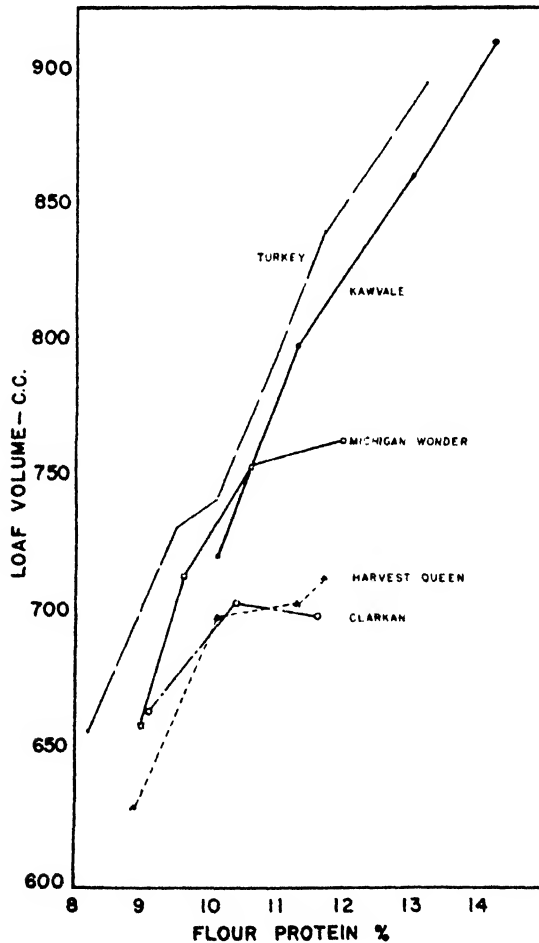


Fig. 3. Loaf volumes obtained by Formula II.

to the hard winter wheats with respect to mixing behavior of the dough. Kawvale is unquestionably differentiated qualitatively from soft wheats. The soft wheats exhibit quite distinctive dough characteristics throughout the range of protein studied, and there seems little doubt that this characteristic may be used to distinguish this class from the hard wheats.

### Wheat-Meal-Fermentation-Time Test

There is no unanimity of opinion as to the usefulness of the wheat-meal-fermentation-time test in differentiating wheats in respect to quality. Pelshenke (1933), Cutler and Worzella (1931, 1933), and Swanson and Dines (1939) have used this method to classify wheats, on the assumption that the length of time is directly related to the

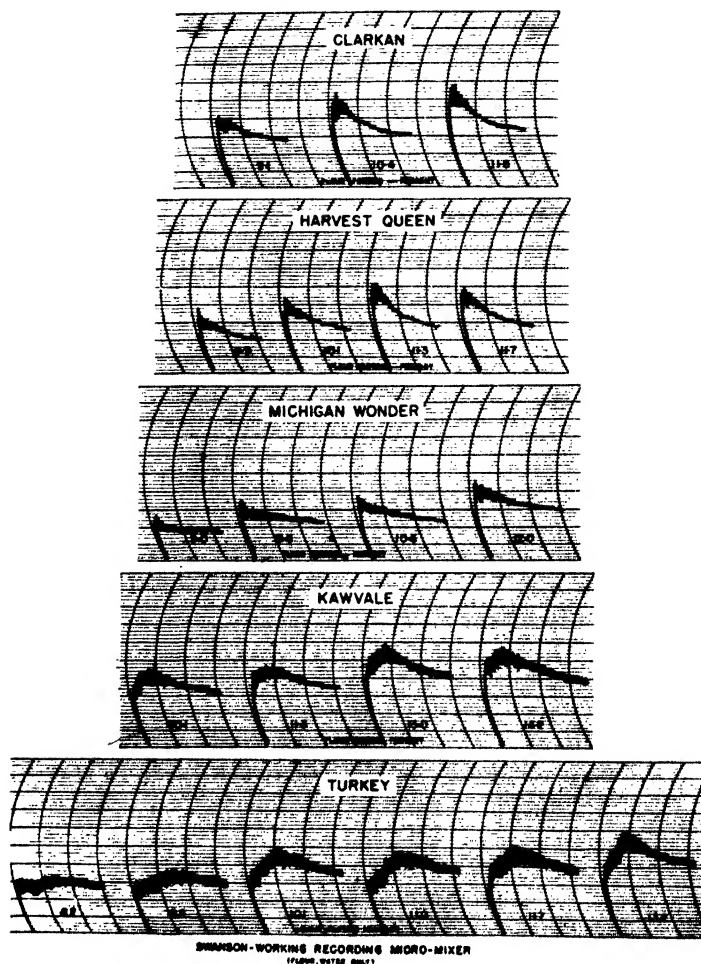


Fig. 4. Curves obtained by the Swanson-Working recording micro-mixer—flour-water doughs.

baking quality. While this may be true over the whole range of bread wheats, which includes a number of distinct classes, there is a considerable amount of overlapping, which makes it difficult to draw very fine distinctions. It is generally accepted that the hard spring wheats exhibit very long times, the hard winter wheats intermediate times, and the soft wheats rather short times. These relative values can be seen in the tables given by Pelshenke (1933) and Geddes (1937.)

The data in Table III for Kansas wheats and the graphs in Figure 5 show that Clarkan, Harvest Queen, and Michigan Wonder tend to be somewhat shorter in time than Kawvale or Turkey. It should be noted, however, that the low-protein Turkey samples exhibited times quite comparable to those of the typical soft wheats. The principal difference between Kawvale and Turkey on the one hand, and Clarkan, Harvest Queen, and Michigan Wonder on the other, is that at protein contents of about 10% the former rise to a substantially

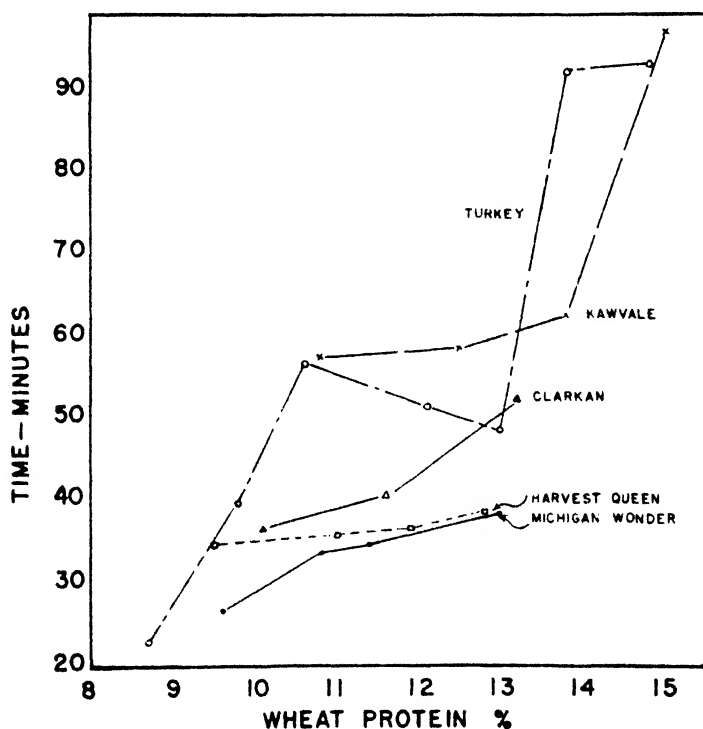


Fig. 5. Relation of wheat-meal-fermentation time to wheat protein content. Number of minutes that elapse from the moment the doughball is put into the water until it starts to disintegrate at the dough-water interface.

higher level of times. Unfortunately no high-protein samples of the latter three varieties were available, and therefore one cannot say that they would not give high time values at high protein contents. From the appearance of the curves, it would seem that Harvest Queen and Michigan Wonder were flattening out, but one can only surmise what might happen in the case of Clarkan, which appears to show increased times throughout its limited range of protein. It might be of interest to add here that Turkey wheat of 17.1% protein content gave a time of 110 minutes, a value comparable to that often obtained with hard spring wheat. There seems no doubt that Kawvale shows the same

magnitude of time as Turkey, and cannot be differentiated from it in this respect.

### Summary and Conclusion

Kawvale, a semi-hard winter variety, appears to be distinctly differentiated from the typical soft varieties Clarkan, Harvest Queen, and Michigan Wonder. It resembles Turkey in wheat-meal-fermentation time, baking performance, and to some extent in mixing-curve characteristics. These similarities were observed over the protein range from 10.7% to 15.0%.

Clarkan, Harvest Queen, and Michigan Wonder were lower in baking quality than either Kawvale or Turkey of comparable protein levels. The latter two were substantially lower in whole-wheat-meal-fermentation time than Turkey, particularly at their upper protein levels which were about 13.0%. They were most distinctly different from Turkey in respect to the character of their mixing curves, showing very rapid development, sharp rapid decline, and early thinning of the curves.

Baking tests of the soft-wheat varieties did not give a linear relation between loaf volume and protein content, the loaf volume tending to fall off at about 10% flour protein. Somewhat better relationship of these variables was obtained with 3 mg. potassium bromate and 6% dry milk solids than with 1 mg. potassium bromate and 4% dry milk solids. This would indicate the need for testing the higher-protein soft-wheat flours with greater dosages of bromate.

### Literature Cited

- Aitken, T. R., and Geddes, W. F.  
1934 The behaviour of strong flours of widely varying protein content when subjected to normal and severe baking procedures. *Cereal Chem.* **11**: 487-504.
- Coleman, D. A., *et al.*  
1930 Milling and baking qualities of world wheats. U. S. Dept. Agr. Tech. Bul. 197.
- Cutler, G. H., and Worzella, W. W.  
1931 A modification of the Saunders test for measuring quality in wheats for different purposes. *Am. Soc. Agron.* **23**: 1000-1009.  
1933 The wheat-meal-fermentation-time test of quality in wheat adapted for small plant breeding samples. *Cereal Chemistry* **10**: 250-262.
- Geddes, W. F.  
1937 The development of new wheat varieties of high milling and baking quality. *Compt. rend. 5th congr. internat. tech. et chim. ind. agr., Sec.* **14**: 148-161.
- and Larmour, R. K.  
1933 Some aspects of the bromate baking test. *Cereal Chem.* **10**: 30-72.
- Larmour, R. K.  
1931 The relation of wheat protein to baking quality. II. Saskatchewan hard red spring wheat crop of 1929. *Cereal Chem.* **8**: 179-189.
- , Working, E. B., Ofelt, C. W.  
1939 Quality tests on hard winter wheats. *Cereal Chem.* **16**: 733-752.

- Pelshenke, P.  
1933 A short method for the determination of gluten quality of wheat. *Cereal Chem.* 10: 90-96.
- Shollenberger, J. H.  
1923 Relation of protein content of wheat to the baking quality of flour as determined from the average results on all commercial and experimentation wheat samples tested by milling investigation—crop years 1915-1920 inclusive. In "Protein and Gluten in Wheat and Flour," Abstracts and References, U. S. D. A. G. I. 12.
- and Clark, J. A.  
1924 Milling and baking experiments with American wheat varieties. U. S. Dept. Agr. Bul. 1183.
- Swanson, C. O.  
1937 Factors which influence results in the wheat-meal-time-fermentation test. *Cereal Chem.* 14: 419-433.
- and Dines, F. T.  
1939 The wheat-meal-time-fermentation test. II. Effect of proteases, protease activators, and protease inhibitors. *Cereal Chem.* 16: 168-177.
- Thomas, L. M.  
1917 A comparison of several classes of American wheats and a consideration of some factors influencing quality. U. S. Dept. Agr. Bul. 557.

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## FAT ACIDITY IN RELATION TO HEATING OF CORN IN STORAGE

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(Read at the Annual Meeting, May 1939)

Spontaneous heating is an ever-present hazard in the storage and shipment of grain. Grain that has been allowed to heat is invariably damaged to some extent. The degree of damage may be imperceptible if the heating is arrested in its very early stages, or it may account for an almost complete loss in commercial value if allowed to progress unchecked.

Spontaneous heating is caused by the heat liberated in the process of respiration, not only of the grain itself but of certain fungi and bacteria proliferating on the surface of the kernels. Whenever conditions are such that heat is produced more rapidly than it is lost by conduction and radiation, the temperature will rise; and since within certain limits the rate of respiration increases with rise in temperature, spontaneous heating once started may become rapidly accelerated.

The rate of respiration, and hence the rate at which heat is developed, depends upon the moisture content of the grain, the temperature, the available oxygen supply, and upon certain characteristics of the grain itself which are not completely understood. Bailey (1921) and Bailey and Gurjar (1918) have made a careful study of the relation of moisture and temperature to rate of respiration in corn and wheat.

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These authors have shown that in the case of damp wheat not only the actual moisture content but the length of time the grain has been stored in a damp condition influence the rate of respiration. They also have shown that grain that has been damaged from various causes respire at a more rapid rate than does sound grain at the same temperature and moisture content.

In commercial practice it has been observed repeatedly that different lots of grain of equal moisture content often display quite different tendencies to heat under similar conditions of storage. Grain having a moisture content that would ordinarily be considered at a safe level for storage sometimes heats within a relatively short time. Other lots of grain at considerably higher moisture levels may remain cool and sweet for an extended period of storage. Obviously, some method for determining this difference in condition between different lots of grain stored under comparable conditions, which would enable storage behavior to be predicted with greater accuracy than can be done from the moisture content alone, would be of considerable practical value.

### Experimental

It has been shown by Zeleny and Coleman (1938, 1939) that the fat acidity of grain, particularly of corn, is a more reliable measure of its degree of soundness than other available chemical or physical tests. In order to test experimentally the value of this determination as a measure of storage behavior, 122 samples of commercial corn, ranging in moisture content from 15.5% to 27.3%, were obtained from Federal Grain Supervision offices in different parts of the country. Cracked corn and foreign material were removed from the samples by appropriate sieving, and representative portions of the clean corn were taken for moisture and fat-acidity determinations. Moisture determinations were made by the water-oven method specified by the Official Grain Standards of the United States (1935). Fat-acidity determinations were made by the method of Zeleny and Coleman (1938, 1939) and were expressed in terms of milligrams of potassium hydroxide required to neutralize the free fatty acids extracted from 100 g. of corn, calculated to a dry-matter basis.

For the heating tests one-quart vacuum bottles were nearly filled with corn, thermometers were inserted to about the middle of the bottles, and loose wads of cotton were inserted in the necks, thus insuring a sufficient interchange of gases to support respiration. The corn, bottles, and thermometers were all preheated to 90°F. before starting the tests. The bottles after filling were placed in an incubator regulated at 90°F. Temperature readings were taken at appropriate intervals, depending on the rate of temperature increase. Rate of heating

was expressed in terms of the number of degrees Fahrenheit increase in temperature above 90°F. per 24 hours. In the case of samples which heated less than 5°F. above 90°F. in 72 hours, the average increase in temperature per 24 hours for the first 72 hours was taken as the rate of heating. For samples which heated more than 5°F. above 90°F. in 72 hours, the rate of heating per 24 hours was calculated from the time required for the sample to heat from 90°F. to 95°F., since at temperatures above 95°F. heat losses were sufficiently great to make temperature readings an unreliable index of the heat produced. In Table I are listed the values for moisture content, fat acidity, and rate of heating obtained for the 122 samples included in this study.

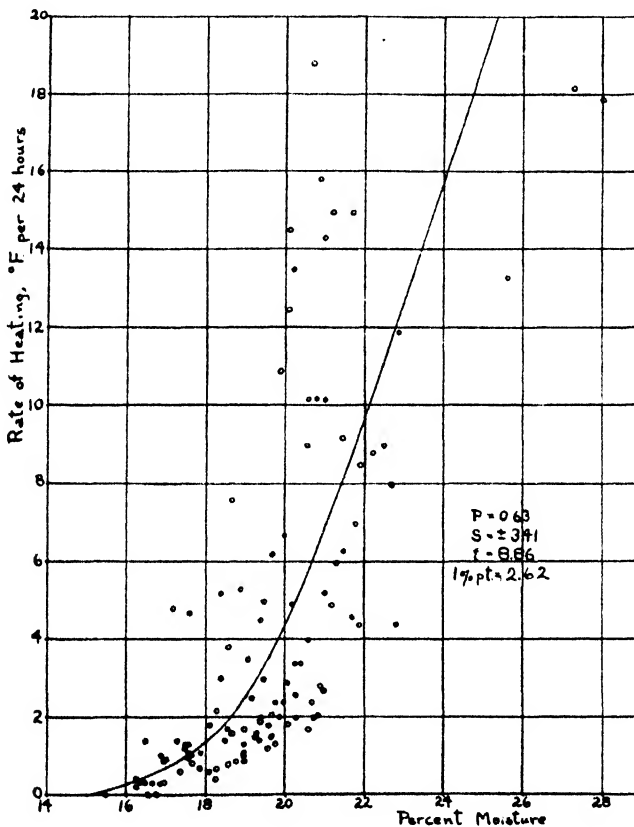


Fig. 1. Relation between moisture content and rate of heating in experimental storage of 122 samples of corn.

### Interpretation of Results

In Figure 1 is shown the relationship between moisture content and rate of heating for the series of samples under investigation. Whereas a general relationship between these two factors is evident, it is obviously not possible to predict reliably the rate of heating of any given

sample from its moisture content. At any given moisture level, however, it may be shown that the rate of heating tends to increase for increasing values of fat acidity. Thus when the rate of heating is plotted against the quantity  $M + .05F$ , where  $M$  is the percentage of moisture and  $F$  is the fat-acidity value (Fig. 2), we obtain a much closer relationship than we did between rate of heating and moisture alone.

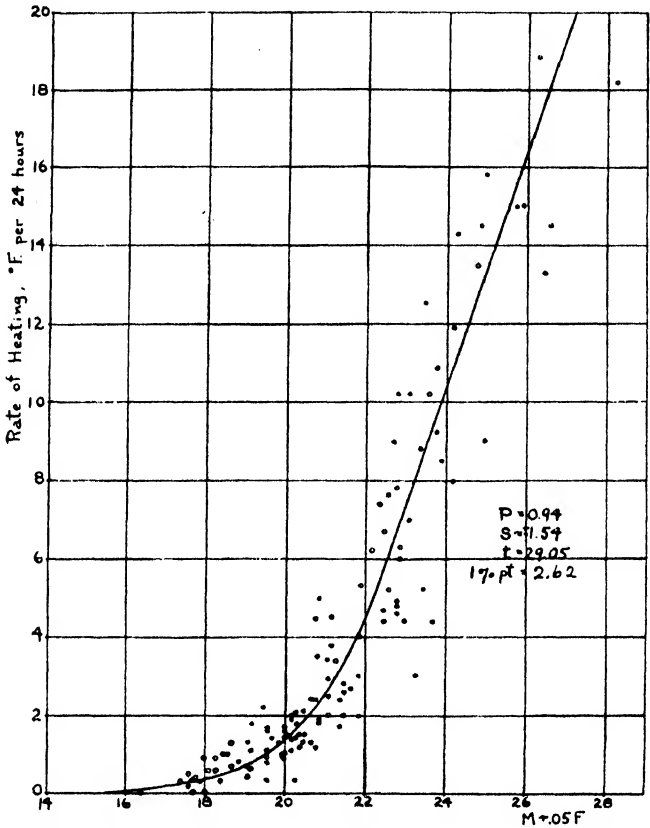


Fig. 2. Relation between  $M + .05F$  (where  $M$  is the moisture content and  $F$  is the fat acidity value) and rate of heating in experimental storage of 122 samples of corn.

Statistical analysis of these relationships yields the following results:

	Index of cor- relation	Standard error of estimate °F.	<i>t</i>	1% point
Rate of heating and moisture (Fig. 1)	0.63	± 3.41	8.86	2.62
Rate of heating and $M + .05F$ (Fig. 2)	0.94	± 1.54	29.05	2.62

It is evident from these values that in any series of corn samples similar to the series under investigation, the rates at which the samples will undergo spontaneous heating under controlled conditions similar to those herein employed, may be predicted with more than twice the

TABLE I

MOISTURE CONTENT, FAT ACIDITY, AND ACTUAL AND PREDICTED RATES OF  
SPONTANEOUS HEATING OF 122 SAMPLES OF CORN

Mois- ture	Fat acidity	$H^1$	$H_M^2$	$H_M - H$	$H_{MF}^3$	$H_{MF} - H$
%		° F.	° F.	° F.	° F.	° F.
15.5	18	0.0	0.1	+0.1	0.1	+0.1
16.3	26	0.2	0.4	+0.2	0.2	0.0
16.3	30	0.4	0.4	0.0	0.3	-0.1
16.4	20	0.3	0.4	+0.1	0.2	-0.1
16.5	29	0.3	0.4	+0.1	0.3	0.0
16.5	77	1.4	0.4	-1.0	1.6	+0.2
16.6	29	0.0	0.5	+0.5	0.3	+0.3
16.7	20	0.3	0.5	+0.2	0.3	0.0
16.7	32	0.6	0.5	-0.1	0.4	-0.2
16.7	18	0.5	0.5	0.0	0.2	-0.3
16.8	19	0.0	0.6	+0.6	0.3	+0.3
16.9	31	0.3	0.6	+0.3	0.4	+0.1
16.9	63	1.0	0.6	-0.4	1.3	+0.3
17.0	52	0.3	0.7	+0.4	1.0	+0.7
17.0	66	0.3	0.7	+0.4	1.6	+1.3
17.0	27	0.9	0.7	-0.2	0.4	-0.5
17.0	20	0.9	0.7	-0.2	0.3	-0.6
17.2	112	4.8	0.8	-4.0	6.9	+2.1
17.3	48	1.4	0.9	-0.5	1.1	-0.3
17.4	15	0.6	0.9	+0.3	0.4	-0.2
17.5	65	1.2	1.0	-0.2	2.2	+1.0
17.5	24	1.3	1.0	-0.3	0.5	-0.8
17.6	19	1.0	1.1	+0.1	0.5	-0.5
17.6	20	1.0	1.1	+0.1	0.5	-0.5
17.6	41	1.1	1.1	0.0	1.0	-0.1
17.6	47	1.3	1.1	-0.2	1.3	0.0
17.6	99	4.7	1.1	-3.6	6.0	+1.3
17.7	24	0.8	1.2	+0.4	0.6	-0.2
17.9	17	0.7	1.3	+0.6	0.5	-0.2
17.9	27	1.1	1.3	+0.2	0.8	-0.3
18.0	23	1.3	1.4	+0.1	0.7	-0.6
18.1	23	0.6	1.5	+0.9	0.8	+0.2
18.1	23	1.8	1.5	-0.3	0.8	-1.0
18.3	17	0.4	1.7	+1.3	0.7	+0.3
18.3	17	0.7	1.7	+1.0	0.7	0.0
18.3	25	2.2	1.7	-0.5	1.0	-1.2
18.3	98	4.0	1.8	-2.2	8.1	+4.1
18.4	103	5.2	1.8	-3.4	8.9	+3.7
18.5	30	1.4	1.9	+0.5	1.4	0.0
18.6	21	0.8	2.0	+1.2	1.0	+0.2
18.6	21	1.7	2.0	+0.3	1.0	-0.7
18.6	52	3.8	2.1	-1.7	2.8	-1.0
18.7	18	1.6	2.2	+0.6	1.0	-0.6
18.7	78	7.6	2.2	-5.4	6.3	-1.3
18.8	16	0.9	2.3	+1.4	1.0	+0.1
18.9	60	5.3	2.5	-2.8	4.3	-1.0

<sup>1</sup> Observed rate of heating, ° F. per 24 hours.

<sup>2</sup> Rate of heating predicted from moisture content (Fig. 1).

<sup>3</sup> Rate of heating predicted from  $M + .05F$  (Fig. 2).

TABLE I—*Continued*

Mois- ture	Fat acidity	$H^1$	$H_M^2$	$H_M - H$	$H_{MF}^3$	$H_{MF} - H$
%		° F.	° F.	° F.	° F.	° F.
19.0	21	0.9	2.6	+1.7	1.4	+0.5
19.0	25	1.1	2.6	+1.5	1.5	+0.4
19.0	31	1.3	2.6	+1.3	1.8	+0.5
19.0	21	1.7	2.6	+0.9	1.4	-0.3
19.0	20	1.0	2.6	+1.6	1.4	+0.4
19.1	34	3.5	2.8	-0.7	2.2	-1.3
19.2	39	2.5	3.0	+0.5	2.7	+0.2
19.3	17	1.5	3.1	+1.6	1.5	0.0
19.3	14	1.6	3.1	+1.5	1.4	-0.2
19.4	16	1.4	3.3	+1.9	1.5	+1.0
19.4	16	1.9	3.3	+1.4	1.5	-0.4
19.4	16	2.0	3.3	+1.3	1.5	-0.5
19.4	29	4.5	3.3	-1.2	2.2	-2.3
19.4	37	4.5	3.3	-1.2	2.8	-1.7
19.5	20	2.1	3.5	+1.4	1.8	-0.3
19.5	18	1.5	3.5	+2.0	1.7	+0.2
19.5	49	3.0	3.5	+0.5	4.3	+1.3
19.5	28	5.0	3.5	-1.5	2.4	-2.6
19.6	16	1.2	3.7	+1.5	1.7	+0.5
19.6	14	1.8	3.7	+1.9	1.6	-0.2
19.7	16	1.5	3.9	+2.4	1.8	+0.3
19.7	13	2.1	3.9	+1.8	1.6	-0.5
19.7	51	6.2	3.9	-2.3	5.0	-1.2
19.8	19	1.3	4.1	+2.8	2.1	+0.7
19.8	18	2.4	4.1	+1.7	2.1	-0.3
19.9	78	10.9	4.3	-6.6	9.9	-1.0
19.9	20	1.9	4.3	+2.4	2.4	+0.5
19.9	20	2.4	4.3	+1.9	2.4	0.0
20.0	17	2.4	4.5	+2.1	2.2	-0.2
20.0	51	6.7	4.5	-2.2	6.0	-0.7
20.0	61	4.4	4.5	+0.1	7.5	+3.1
20.0	133	14.5	4.5	-10.0	18.2	+3.7
20.1	17	1.8	4.7	+2.9	2.4	+0.6
20.1	21	2.9	4.7	+1.8	2.7	-0.2
20.1	69	12.5	4.7	-7.8	9.0	-3.5
20.1	96	14.5	4.7	-9.8	13.1	-1.4
20.2	40	4.9	5.0	+0.1	5.0	+0.1
20.2	92	13.5	5.0	-8.5	12.8	-0.7
20.3	17	2.0	5.2	+3.2	2.7	+0.7
20.3	25	2.6	5.2	+2.6	3.3	+0.7
20.3	16	3.4	5.2	+1.8	2.7	-0.7
20.4	19	3.4	5.5	+2.1	3.0	-0.4
20.6	17	1.7	6.0	+4.3	3.2	+1.5
20.6	27	4.0	6.0	+2.0	4.3	+0.3
20.6	42	9.0	6.0	-3.0	6.6	-2.4
20.6	45	10.2	6.0	-4.2	6.9	-3.3
20.7	14	2.4	6.3	+3.9	3.2	+0.8
20.7	113	18.8	6.3	-12.5	17.3	-1.5
20.8	14	2.0	6.6	+4.6	3.3	+1.3
20.8	22	2.0	6.6	+4.6	4.3	+2.3
20.8	33	7.4	6.6	-0.8	5.7	-1.7
20.8	46	10.2	6.6	-3.6	7.8	-2.4
20.8	41	7.8	6.6	-1.2	6.9	-0.9
20.9	12	2.8	7.0	+4.2	3.3	+0.5
20.9	83	15.8	7.0	-8.8	13.5	-2.3

TABLE I—*Continued*

Mois- ture	Fat acidity	$H^1$	$H_M^2$	$H_M - H$	$H_{MF}^3$	$H_{MF} - H$
%		° F.	° F.	° F.	° F.	° F.
21.0	14	2.7	7.1	+4.4	3.8	+1.1
21.0	33	5.2	7.1	+1.9	6.3	+1.1
21.0	53	10.2	7.1	-3.1	9.3	-0.9
21.0	66	14.3	7.1	-7.2	11.3	-3.0
21.2	32	4.9	7.6	+2.7	6.9	+2.0
21.2	93	15.0	7.6	-7.4	15.8	+0.8
21.3	32	6.0	7.9	+1.9	7.2	+1.2
21.5	28	6.3	8.4	+2.1	7.2	+0.9
21.5	46	9.2	8.4	-0.8	9.9	+0.7
21.7	18	4.6	8.7	+4.1	6.9	+2.3
21.7	84	15.0	8.7	-6.3	16.1	+1.1
21.8	27	7.0	9.3	+2.3	7.8	+0.8
21.9	13	4.4	9.6	+5.2	6.0	+1.6
21.9	41	8.5	9.6	+1.1	10.1	+1.6
22.2	25	8.8	10.5	+1.8	8.7	-0.1
22.5	50	9.0	11.4	+2.4	13.5	+4.5
22.7	31	8.0	12.0	+4.0	11.1	+3.1
22.8	18	4.4	12.3	+7.9	9.6	+5.2
22.9	27	11.9	12.6	+0.7	11.1	-0.8
25.6	19	13.3	21.2	+7.9	17.9	+4.6
27.3	21	18.2	26.2	+8.0	23.1	+4.9

accuracy when both moisture content and fat acidity are considered than when moisture content is considered alone. This is further illustrated by the data in the last four columns of Table I showing the rates of heating as predicted from the moisture content and as predicted from both fat acidity and moisture, together with the differences between predicted and actual rates of heating.

Figure 3 shows graphically the relationships between fat acidity, moisture content, and rate of heating, which apply under the experimental conditions used. These curves are derived from the curve in Figure 2 by substituting specific values for  $M$  and  $F$ , and serve to illustrate the value of the fat-acidity determination as an index of storage behavior. Thus it may be seen, for example, that under carefully controlled conditions corn with a moisture content of 17% and a fat-acidity value of 100 can be expected to heat in storage as rapidly as corn with a moisture content of 21% and a fat-acidity value of 20.

It is not contended that the presence of free fatty acids as such in corn in any way affects its rate of respiration, and thus its tendency to heat. Fat acidity should be considered merely as a useful index of more obscure chemical, physical, or biological changes occurring during the deterioration of corn that appear to stimulate its rate of respiration. It must be conceded that the rather limited number of samples in-

cluded in this study does not preclude the possibility that under certain conditions respiration of corn at a given moisture level might be stimulated without an increase in fat acidity, or that fat acidity might increase greatly without a corresponding increase in respiration. No such instances, however, have appeared in this study.

It should be emphasized that the curves in Figure 3 may be used for predicting rates of heating only for corn in experimental storage under a given set of conditions, and that it is not probable that the same curves would be applicable to corn in commercial storage. It is reasonable to expect, however, that under any normal storage condi-

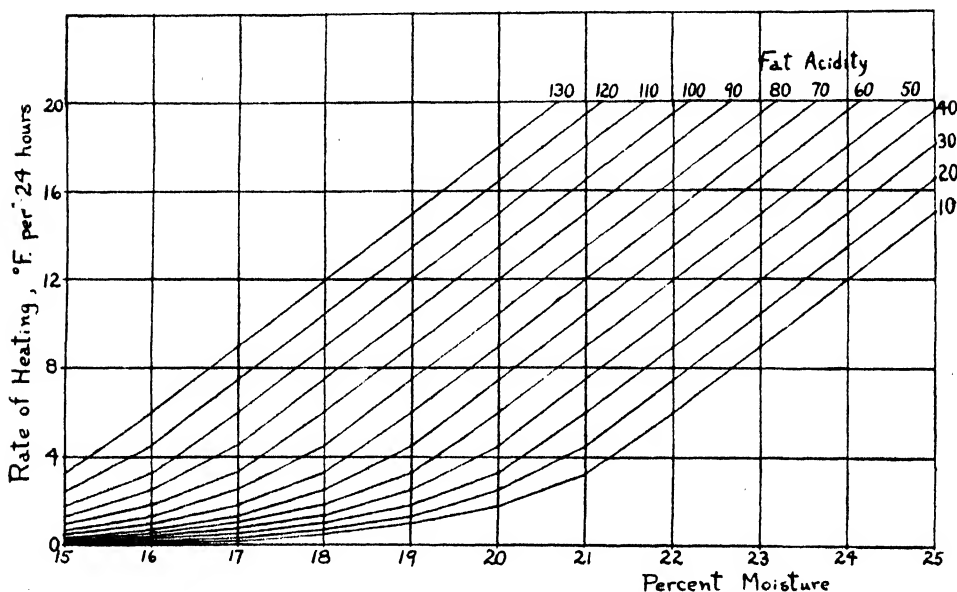


Fig. 3. Relation between moisture content, fat acidity, and rate of heating for corn in experimental storage.

tions, either commercial or experimental, analogous relationships between moisture content, fat acidity, and rate of heating will hold, and that low-acidity corn may be as good a commercial storage risk as high-acidity corn containing as much as 4% less moisture. Safe fat-acidity limits for the commercial storage of corn at different moisture levels cannot be determined solely by laboratory experimentation, but only through the extensive practical application of the fat-acidity test to corn in storage and the observation of its relationship to storage behavior.

It is anticipated that such data will gradually be accumulated through the cooperation of interested commercial laboratories, and that this cooperative effort may lead eventually to the establishment of useful fat-acidity limits at different moisture levels for the safe commercial storage of corn and possibly of other cereal grains.

## Literature Cited

- Bailey, C. H.  
1921 Respiration of shelled corn. Minn. Agr. Exp. Sta. Tech. Bul. 3.  
— and Gurjar, A. M.  
1918 Respiration of stored wheat. J. Agr. Res. 11: 685-713.  
U. S. Department of Agriculture  
1935 Air-oven and water-oven methods specified in the United States Official Grain Standards for determining the moisture content of grain. Bur. Agr. Econ., Serv. and Reg. Announc. No. 147.  
Zeleny, L., and Coleman, D. A.  
1938 Acidity in cereals and cereal products, its determination and significance. Cereal Chem. 15: 580-595.  
1939 The chemical determination of soundness in corn. U. S. Dept. Agr. Tech. Bul. 644.

PLASTICITY OF DOUGHS <sup>1</sup>OLOF E. STAMBERG <sup>2</sup> and C. H. BAILEY

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(Read at the Annual Meeting, May 1939)

Various methods have been employed for determining the relative plasticity of doughs, and particularly as this property is related to the measurement of the water absorption of flour and other dough ingredients. Several of the devices employed for that purpose are described by Markley and Bailey (1938). Sharp (1926) used a pressure plastometer for studying the rate of flow through a fine capillary of flour-in-water suspensions. A yield value was observed with 19% or more of flour by weight, thus indicating a plastic system, but Sharp did not use concentrations over 33% flour and hence did not operate within the limits of plasticity of doughs ordinarily encountered in bread doughs. St. John and Bailey (1929) also used a pressure plastometer and studied the rate of flow of flour-water suspensions. They likewise employed suspensions much higher in water content than ordinary doughs.

Halton and Scott Blair (1936b) extruded doughs from a "gun," using a weight of seven pounds in a study of various physical properties of doughs. Halton and Fisher (1938) describe a dough plastometer which measures the rate of extrusion through an aperture. Pressure was applied to the dough by a piston resting on the dough surface. This piston was attached to a weight suspended by a fine wire which passed through the aperture in the bottom of the cylinder in which the dough was contained.

The instrument described here is based on the principles of the pressure plastometers and intended for doughs in a range of plasticity generally used for baking.

<sup>1</sup> Paper No. 1749, Scientific Journal Series, Minnesota Agricultural Experiment Station.<sup>2</sup> American Dry Milk Institute Research Associate.



### The Apparatus

Figure 1 is a diagram of the apparatus. The main air-pressure valve is shown at *A*, and *B* is a diaphragm-type pressure regulator used to maintain a constant pressure as indicated by *C*, the mercury manometer.

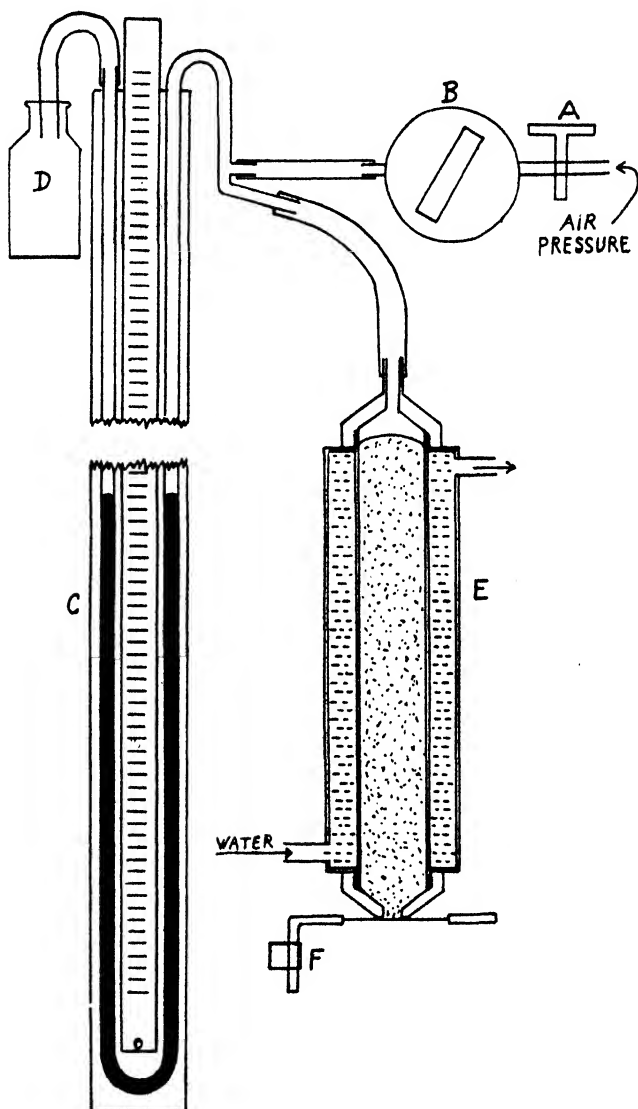


Fig. 1. Diagram of the pressure plastometer.

The extrusion apparatus (*E*) is provided with a water jacket similar to a Liebig condenser through which water at constant temperature from a water thermostat is circulated by means of a centrifugal pump. The dough cylinder is 22 mm. in diameter and 170 mm. long, the

jacket cylinder 44 mm. in diameter and about 140 mm. long. Both cylinders are made from brass pipe. The top and the bottom of the dough cylinder are threaded and can easily be removed for filling and also for cleaning by means of a tight-fitting plunger. The opening at the bottom is exactly 6 mm. in diameter and the thickness of the bottom cap through which this aperture is bored is 6 mm.

A fine wire (*F*) is mounted on a frame so that it can swing back and forth for cutting the extruded dough sharply at the aperture. The bottle (*D*) receives any overflow of mercury which results from inadvertently increasing the pressure unduly when adjusting the valves to the desired pressures.

Doughs were made from 100 g. of flour (13.5% moisture), 2 g. of salt and the necessary water, and were mixed for two minutes in the Hobart-Swanson mixer. About 75 g. of dough were required to fill the apparatus, and this was done within 30–45 seconds after the mixing was completed. The dough was rolled into a long cylinder, immediately introduced through the top opening of the dough cylinder, and then packed down with the plunger. A series of preliminary tests indicated that the results were most constant when the dough was allowed to rest in the cylinder for five to six minutes. The rate of flow was slower with less than five minutes of rest in the cylinder, but it was practically constant after five and up to 12 minutes. It was necessary to introduce the dough into the cylinder at a temperature within one degree of that required for the test. The temperature of the dough could be determined by removing the rubber tubing from the upper cap and inserting a thermometer. With the aperture of 6 mm. a pressure of 500 mm. of mercury was found to be most satisfactory with doughs of ordinary plasticity.

After the five- to six-minute resting period which followed the introduction of the dough into the cylinder, the pressure was applied through valve *A* and adjusted with valve *B*. The latter had previously been set for the desired pressure and only minor adjustments were necessary after the pressure was applied. About four to five grams of dough was allowed to flow out, after which the dough was cut at the same instant that a stop-watch was started. The dough was allowed to flow for one minute into a tared pan, and the quantity of extruded dough was immediately weighed to the nearest centigram.

It is quite important that four to five grams of dough be allowed to flow out of the cylinder before the test is started. Only the rate of flow for the first minute thereafter was used, since the second minute of flow did not afford close replicates and particularly with the slacker doughs when the air sometimes broke through.

Closest replicates were obtained when the plasticity of the dough was within the limits of flow of two to ten grams per minute. Slacker doughs gave best results by measuring the flow for one-half minute and recording double that weight for the equivalent of the standard one-minute flow. The most difficult operation in securing replicated results in close agreement involved the control of the degree of compactness of the dough as it was rolled out to be placed in the apparatus. Air pockets in the dough must be carefully avoided. After some experience the results can be replicated quite satisfactorily by the operator.

### Experimental

The effects of temperature and pressure upon the plasticity of dough were studied with a flour containing 11.60% crude protein ( $N \times 5.7$ ) and a constant proportion of water equivalent to 60%. The pressure used was 500 mm. of mercury in the study of temperature variations, and the temperature of the dough was 30°C. in the study of pressure variations. The results of these tests are shown graphically in Figure 2. A variation in temperature of one degree changed the rate of flow about 0.29 g. per minute, thus indicating that temperature control was essential to secure comparable results. A variation in pressure of 10 mm. changed the rate of flow only about 0.17 g. per minute, and the pressure was easily controlled to within  $\pm 2$  mm. during a test. It accordingly appeared that the variability occasioned by such a range of pressure would be of small consequence. In the instances of both series of studies the rate of flow in grams per minute was practically a linear function of temperature and of pressure respectively.

Employing three flours: *A*, with 8.32% crude protein (13.5% moisture); *B*, with 11.60% crude protein; and *C*, with 15.50% crude protein, the rate of flow was studied in doughs made with varying proportions of water. The pressure used was 500 mm. and the temperature 30°C. The results of these tests are shown by the graph in Figure 3. The lower half records the rate of flow in grams per minute plotted against the percentage of water used. It was found that when the logarithm of the rate of flow was plotted against the percentage of water used, as recorded in the upper half of Figure 3, straight lines were obtained. Thus the relative absorption of different flours can be compared by means of these straight lines and two points accurately determined can establish them, although three points are desirable.

When dry-milk solids was added to doughs in the proportion of six grams per 100 grams of flour, the logarithmic lines of the milk doughs were parallel to those of the milk-free doughs. The distance between these parallel lines varied according to the absorption of the dry milk solids.

A farinograph was not available at the time the pressure plastometer was used, but samples of flours *A*, *B*, and *C* were stored at room temperature in hermetically sealed containers for five months, when farinograms were made. The absorption was assumed to correspond to a rate of flow of 0.7 on the logarithmic scale, which is equivalent to

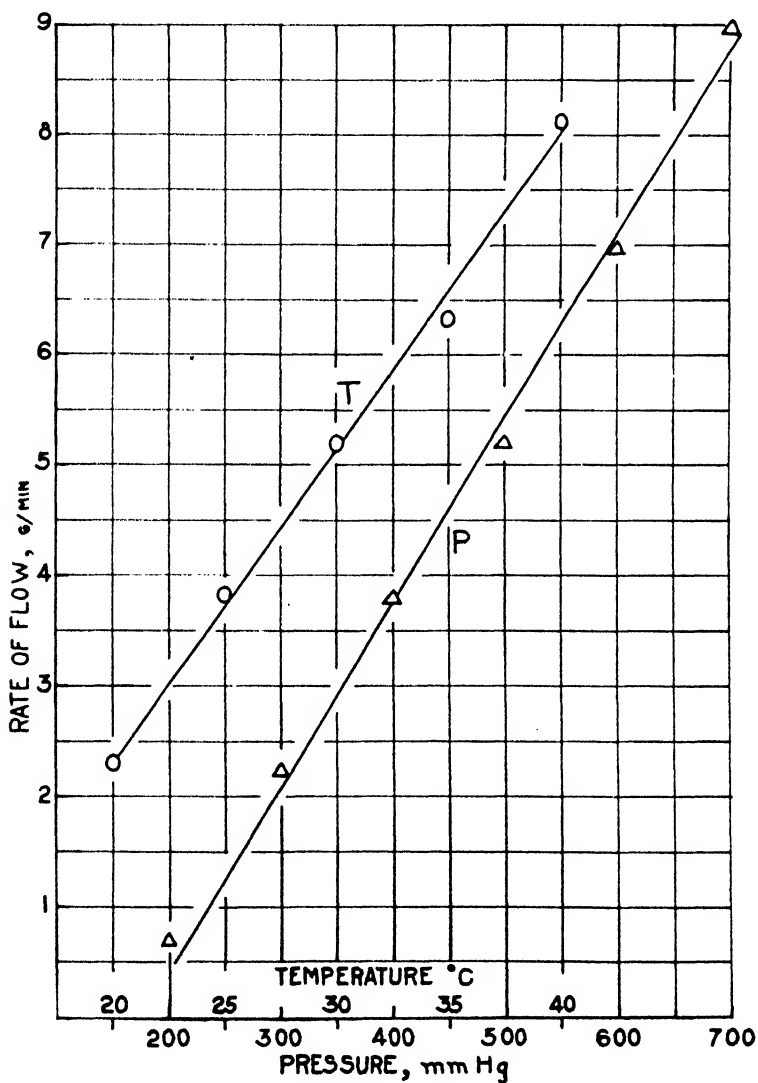


Fig. 2. Effect of temperature (*T*) and pressure (*P*) on the rate of flow of flour doughs.

5.01 g. of dough per minute. The data from the resulting farinograms are shown in Table I.

The farinograph tests indicate that the absorptions which gave the same rate of flow with the pressure plastometer resulted in different minimum mobilities as measured on the farinograph. One probable

reason for this is that a constant mixing time was employed for the doughs used in the pressure plastometer, while the minimum mobility values from the farinograph were based on the mixing time necessary to reach the minimum mobility, which was variable, as shown in Table I. No tests were carried out for this study with variable mixing times.

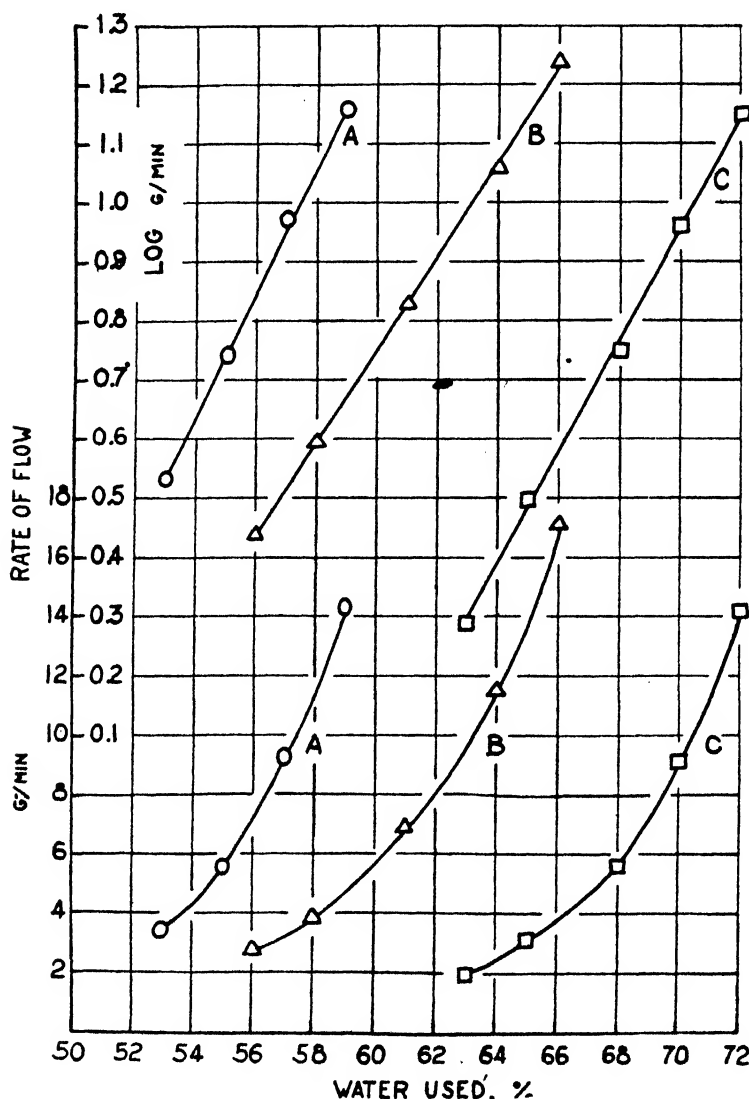


Fig. 3. Effect of proportion of water used on the rate of flow of doughs using three flours: A, with 8.32% protein; B, with 11.60% protein; and C, with 15.50% protein.

In using the pressure plastometer it was observed that in extruding the dough made from the soft wheat flour A, with 8.32% protein, the cylinder of dough retained practically the diameter of the aperture. In the case of the hard-wheat flours, B with 11.60% protein and C with

TABLE I  
RESULTS OF FARINOGRAPH TESTS

Flour	Absorption	Mixing time to reach minimum mobility	Minimum mobility, in farinograph units
A	54.5	7	410
B	59.0	11	460
C	67.5	17	480

15.50% protein, the extruded dough cylinder increased in diameter as it emerged from the aperture.

Halton and Scott Blair (1936a) also observed that in extruding cylinders of dough from their dough gun, the dough cylinder swelled, "this swelling being in general greater for good than for poor quality flours." They plotted graphs showing the progressive changes in viscosity and rigidity modulus with increasing proportions of water in doughs prepared from strong and weak flours. The rigidity modulus increased at a substantial rate with increasing percentages of water in the dough. Moreover the rigidity modulus was increased more per unit of water added in the instance of a weak flour than with the strong flour. This might be interpreted to imply less sensitivity in terms of rigidity modulus in the instance of the strong flour, and hence a lesser tendency to lose the capacity to swell when the dough cylinder is extruded through an aperture. Their data also indicate that the rigidity modulus ( $n$ ) of the weak flour doughs is higher at any level of viscosity ( $\eta$ ) than the strong flour doughs and the ratio between these two physical properties,  $\eta/n$ , or relaxation time, may be a characteristic of flour strength.

Thus it is possible that different physical properties are affecting the rate of flow of the low-protein soft-wheat flour *A*, as compared to the higher-protein hard-wheat flours *B* and *C*. The described instrument may prove useful for the determination of relative absorptions of flours, or other dough ingredients.

### Summary

A plastometer of the extrusion type was designed and constructed for use in the study of the plasticity of bread doughs. The apparatus was so constructed that doughs maintained at a constant temperature were extruded through a 6-mm. aperture from a cylinder by means of air pressure equivalent to 500 mm. of mercury. Rate of flow per minute was used as an index of the relative plasticity of the doughs. The effect of various pressures and temperatures on the rate of flow was studied, and it was found that small temperature variations may

affect the rate of flow to a greater extent than any probable variations in pressure, thus emphasizing the necessity of precise temperature control in making such measurements.

The logarithm of the rate of flow was a linear function of the proportion of water to flour used in mixing the dough.

The apparatus may be useful in studying relative absorptions of flours and other dough ingredients.

#### Literature Cited

- Halton, P., and Fisher, E. A.  
1938 Dough viscometer. Brit. patent 492,049. Sept. 14, 1938.
- Halton, P., and Scott Blair, G. W.  
1936a A study of some physical properties of flour doughs in relation to their bread-making qualities. *J. Phys. Chem.* **40**: 561-80.  
1936b The relationship between conditions governing rupture and flow in flour doughs. *J. Phys. Chem.* **40**: 811-19.
- Markley, M. C., and Bailey, C. H.  
1938 The colloidal behavior of flour doughs. II. A study of the effects of varying the flour-water ratio. *Cereal Chem.* **15**: 317-26.
- Sharp, P. F.  
1926 Wheat and flour studies. V. Plasticity of simple flour-in-water suspensions. *Cereal Chem.* **3**: 40-56.
- St. John, J. L., and Bailey, C. H.  
1929 The effect of dry skim milk upon the water absorption of doughs and the plasticity of flour suspensions. *Cereal Chem.* **6**: 140-50.

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## THE PRESSUREMETER IN THE STUDY OF YEAST

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(Read at the Annual Meeting, May 1939)

A recognized need of the cereal technologist is a convenient method of testing the uniformity and the characteristics of various commercial yeasts. Since the very wide acceptance of the pressuremeter designed by Sandstedt and Blish (1934) makes this equipment generally available, its suitability as a device for testing yeast was studied.

A method for determining the carbon dioxide produced from maltose in a liquid medium was reported by Cook and Malloch (1930). This method has the obvious disadvantage that yeast does not react the same way in a liquid medium as it does in a dough. Many methods have been devised to measure the gas given off by a dough rather than the total gas produced, which includes the gas retained and the gas evolved during fermentation. Bailey and Johnson (1924) collected the gas given off in an inverted burette. C. W. Brabender (1934) devised the fermentograph. Sullivan and Near (1935) published observations on the variability in gassing strengths of various yeasts. They found these data correlated very well with the gas produced during the proofing period of the baking test.

The work of Bohn and Favor (1938) strongly indicated that the pressuremeter has considerable merit as a means of testing yeast uniformity and provides a means of studying different yeasts. With the work of Bohn and Favor as a guide, experimental work was carried out using sucrose and maltose in straight doughs to see which sugar would show the greatest differences between various yeasts and between daily shipments of the same yeast. Following this procedure, the pressuremeter was used on regular straight-dough gassing-power determinations and with a method designed to represent the sponge dough. The data from these two methods were compared with baking-test data recorded during the time the pressuremeter records were being made.

### Experimental

For all experimentation one flour, an unbleached and unmalted commercially milled flour, was used in order to eliminate flour as a variable. This also eliminated the possibility of differences in bleaching action or protease activity during the experiments.

In this type of work it is essential that there be no sugar deficiency so that the yeast activity itself will be the limiting factor in the fermentation. The amount of sugar to be used was determined by the following formula:  $1000 - (\text{gassing power} \times 1.33) = \text{mg. sugar}$ . The gassing power is the fourth-hour pressuremeter reading in mm. mercury. This is a formula suggested by R. M. Sandstedt for determining the amount of sugar to add in an experimental bake to give 10% total sugar in the dough (maltose developed in the dough during a four-hour fermentation and proof + sugar added = 10%). Ten percent total sugar insured a nearly constant excess in all bakes.

Our fourth-hour reading was 289, using the formula:  $1000 - (289 \times 1.33) = 0.6156 \text{ g. of sugar required}$ . This amount of sugar was used throughout the experiments whether maltose or sucrose was used, both in the straight- and sponge-dough methods.

Preliminary work was done to determine the value of pressuremeter determinations for checking yeast uniformity. One-pound cakes of each of four popular yeasts delivered daily were used for these experiments. These four commercial yeasts will be identified as yeasts *A*, *B*, *C*, and *D*.

Table I shows typical data for the fourth-hour readings over a period of 10 days. It may be noted that yeast *D* showed a definite drop on the fourth day. Yeast *D* was baked on the third, fourth, and fifth days and the proof time lengthened 3 minutes on the fourth day. All four yeasts were very uniform in gassing strength from day to day. Baking tests showed that unless a yeast varied over 25 mm.



TABLE I

FOURTH-HOUR PRESSUREMETER READINGS OVER A PERIOD OF TEN DAYS  
(0.3 g. of each of four commercial yeasts, 0.6156 g. sucrose with 10 g. flour and 10 cc. distilled water)

Yeast	Days										Range of readings
	1	2	3	4	5	6	7	8	9	10	
	<i>Millimeters of mercury</i>										
A	450	456	459	453	466	450	442	455	459	449	24
B	485	480	469	488	494	489	477	480	472	483	25
C	433	410	440	435	459	435	442	429	433	445	49
D	507	495	510	455	504	500	498	511	509	502	56

on a single pressuremeter determination, the difference was not noticeable in the loaf volume, but was paralleled by the proof time.

To see if maltose would show wider daily variations in the yeasts it was tried instead of sucrose. The maltose seemed to show wider differences between yeasts but not much difference in daily variations. Therefore for checking a yeast for daily uniformity either sugar could be used with good results.

The averages of ten days' gassing-power results using 0.6156 g. of sucrose, 10 g. of flour, 0.3 g. of yeast and 10 cc. of distilled water appear in Table II. From this table it is apparent that yeast A

TABLE II

AVERAGE OF 10 DAYS' GASSING-POWER RESULTS ON DOUGHS USING FOUR COMMERCIAL YEASTS

Hour	Yeast A		Yeast B		Yeast C		Yeast D	
	Total	Hourly	Total	Hourly	Total	Hourly	Total	Hourly
	<i>Millimeters of mercury</i>							
1	144	144	114	114	101	101	131	131
2	260	116	252	138	224	123	281	150
3	364	104	363	111	332	108	397	116
4	455	91	483	120	437	105	507	110
5	544	89	592	109	544	107	616	110
6	623	79	690	98	646	102	717	101
7	700	77	768	78	721	75	765	48

Formula: 0.6156 g. sucrose, 10 g. flour, 0.3 g. of each yeast, and 10 cc. distilled water.

works very fast the first hour but then slows down very decidedly. Yeast B reaches its peak at the second hour but does not slow down as fast as yeast A. Yeast C is slower at the start but very steady through the sixth hour of fermentation. Yeast D produced gas very rapidly at the start, as did yeast A, but did not drop off nearly as fast and was still producing well at the sixth hour. Considering the fourth hour as the critical time in straight-dough fermentation, these

data show that yeasts *B* and *D* would be producing gas at the greatest rate during the proof time, yeast *C* would be a close third, and yeast *A* would be noticeably slower.

Exactly the same procedure was followed for gassing-power studies using maltose in place of sucrose to learn if it would show greater differences between yeasts. The averages of ten days appear in Table III. Yeast *A* seemed much better able to use maltose than

TABLE III  
AVERAGE OF 10 DAYS' GASSING-POWER RESULTS ON DOUGHS USING FOUR  
COMMERCIAL YEASTS

Hour	Yeast A		Yeast B		Yeast C		Yeast D	
	Total	Hourly	Total	Hourly	Total	Hourly	Total	Hourly
<i>Millimeters of mercury</i>								
1	92	92	97	97	92	92	96	96
2	250	158	258	161	227	135	269	173
3	388	133	394	136	357	130	426	157
4	513	125	528	134	474	117	562	136
5	634	121	659	131	585	111	665	103
6	691	57	699	40	666	81	698	33
7	715	24	724	25	708	42	720	22

Formula: 0.6156 g. maltose, 10 g. flour, 0.3 g. of each yeast, and 10 cc. distilled water.

sucrose, but even with maltose yeasts *B* and *D* were producing more gas during the fourth hour than either of the other two. Yeast *C* using maltose was a poor fourth in gas production. The question then arose, which sugar was giving a true picture? To determine this, a small test bake was run which showed that using proof time as an indication of rate of gas production, both sugars were giving true pictures. When sucrose was added to the doughs, yeast *A* required the longest time to proof to height, and when maltose was used yeast *C* required the longest time to proof to height. Therefore, since sucrose is added to doughs in test baking, it was decided to use sucrose in the gassing-power tests for yeasts. However, in bakeries where malt is used it is quite possible that maltose and sucrose should be used in evaluating yeasts.

At this stage of the experiments, baking tests were run with both the straight-dough and the sponge-dough method, in both cases with sucrose in the formula. For the baking tests, however, bleached flour was used which had been milled at exactly the same time on the same mill as the flour used for the pressuremeter determinations, but which had been taken off the mill after instead of before the bleach. Preliminary baking tests showed a correlation between yeast activity and proofing time. Further investigation showed that variations between

yeasts also affect the characteristics of the finished loaf. The doughs<sup>1</sup> were mixed two minutes on the G-R Swanson dough mixer, scaled into two equal doughs, and placed in the fermentation cabinet at 86°F. for three hours. This temperature was the same as that of the water bath used in all pressuremeter determinations. The doughs were machine-punched twice, machined for panning, proofed to height and baked for 30 minutes in a rotary hearth oven at 425°F. The Thompson Model G Roll Moulder was used for all punches and for pan molding. The average proof time for seven days' baking (not in sequence) appears in Table IV. The pressuremeter indicated these results except for a slightly larger difference between yeasts *B* and *D*. It should be noted that yeast *A* was the last to proof to height each day of the bake and yeast *C* was next to last in proofing to height all but two days.

TABLE IV  
DAILY PROOF TIME OF DOUGHS USING FOUR COMMERCIAL YEASTS

First in proofing to height	Second in proofing to height	Third in proofing to height	Fourth in proofing to height
Yeast <i>C</i> 44½ min.	Yeast <i>D</i> 45 min.	Yeast <i>B</i> 48 min.	Yeast <i>A</i> 50½ min.
<i>C</i> 49½	<i>B</i> 51	<i>D</i> 54	<i>A</i> 58
<i>D</i> 46	<i>B</i> 47	<i>C</i> 48	<i>A</i> 50½
<i>D</i> 49	<i>B</i> 49½	<i>C</i> 50	<i>A</i> 53½
<i>D</i> 52	<i>B</i> 53	<i>C</i> 54	<i>A</i> 58
<i>B</i> 50½	<i>D</i> 53	<i>C</i> 53½	<i>A</i> 57
<i>B</i> 52½	<i>D</i> 53½	<i>C</i> 55	<i>A</i> 57

Average proof time for the seven-day bake: yeast *B* 50.2 min., yeast *D* 50.3 min., yeast *C* 51 min., and yeast *A* 55 min.

The loaves were scored for inside and outside characteristics and were evaluated by giving to the duplicates showing the best development a grade of 1, the next best 2, etc. The result of this composite

TABLE V  
COMPOSITE INTERIOR AND EXTERIOR EVALUATIONS OF THE DUPLICATE LOAVES  
USING FOUR COMMERCIAL YEASTS

	Days						
	1	2	3	4	5	6	7
	Yeasts						
The yeast rating <i>first</i> on loaf characteristics	<i>B</i>	<i>D</i>	<i>D</i>	<i>C</i>	<i>A</i>	<i>A</i>	<i>B</i>
Yeast rating <i>second</i>	<i>D</i>	<i>C</i>	<i>B</i>	<i>B</i>	<i>B</i>	<i>B</i>	<i>D</i>
Yeast rating <i>third</i>	<i>C</i>	<i>B</i>	<i>C</i>	<i>D</i>	<i>C</i>	<i>D</i>	<i>C</i>
Yeast rating <i>fourth</i>	<i>A</i>	<i>A</i>	<i>A</i>	<i>A</i>	<i>D</i>	<i>C</i>	<i>A</i>

scoring on the seven days' bake appears in Table V. Giving 3 points to the yeast rating 1, 2 points to the yeast rating 2, and 1 point to the yeast rating 3 for each bake, we find the value of yeast *A* to

<sup>1</sup> Formula: 200 g. flour (15% moisture basis, undiastated), 12 g. sugar, 3½ g. salt, 6 g. yeast, 4 g. liquid shortening.

be 6, yeast *B* 15, yeast *C* 9, and yeast *D* 12. Here again is a correlation with the rate of gas production, for the pressuremeter showed yeast *B* as producing most gas during the fourth hour, yeast *D* second largest production, yeast *C* next, and yeast *A* producing the least. It should be noted that rate of gas production is here correlated with texture and crust characteristics, possibly because of the proper development of the dough under this set of conditions.

TABLE VI  
VOLUMES OBTAINED DURING SEVEN DAYS' BAKES USING FOUR COMMERCIAL YEASTS

Yeast <i>D</i> 685 cc.	Yeast <i>B</i> 683 cc.	Yeast <i>C</i> 675 cc.	Yeast <i>A</i> 660 cc.
<i>A</i> 670	<i>C</i> 660	<i>B</i> 657	<i>D</i> 653
<i>D</i> 680	<i>B</i> 675	<i>A</i> 665	<i>C</i> 655
<i>C</i> 675	<i>B</i> 665	<i>D</i> 660	<i>A</i> 645
<i>D</i> 670	<i>A</i> 668	<i>C</i> 662	<i>B</i> 645
<i>B</i> 690	<i>D</i> 685	<i>C</i> 680	<i>A</i> 663
<i>D</i> 675	<i>B</i> 670	<i>C</i> 660	<i>A</i> 653

The loaf volumes for the seven bakes appear in Table VI. Applying the same system of evaluation, the volume scores, yeast *A* rates 6 points, yeast *B* 12, yeast *C* 9, and yeast *D* 12. Note that the rate of gas production the fourth hour does have a definite effect on the loaf volumes. Yeast *D*, which was producing gas at the greatest rate during the fourth hour, had the largest loaf volume 4 out of 7 days' bakes, yeast *B*, which produced gas next in line, was second in loaf volume 4 out of 7 days' bakes, yeast *C* was third 4 out of 7 and yeast *A* was last 4 out of 7.

TABLE VII  
COMBINED DATA OF PRESSUREMETER, PROOF TIME, LOAF VOLUME AND COMPOSITE SCORE IN THE STRAIGHT-DOUGH PROCEDURE

Yeast	Average fourth-hour gas production <sup>1</sup>	Average proof time <sup>2</sup>	Average loaf volume	Average composite score
	<i>mm.</i>	<i>min.</i>	<i>cc.</i>	
<i>A</i>	91	55	660.5	Fourth place
<i>B</i>	120	50.2	669	First place
<i>C</i>	105	51	666.7	Third place
<i>D</i>	110	50.3	672.5	Second place

<sup>1</sup> The gassing-power data are a ten-day average.

<sup>2</sup> The baking data are a seven-day average.

Table VII is presented to show the pressuremeter data and the baking data in a composite picture. Here we find that the fourth-hour gas production in the pressuremeter gave a good picture of what the four yeasts would do in straight-dough baking. It should be noted that the baking was alternated between the two authors who kept

their own data until all seven bakes were finished. It should also be noted that the texture and loaf characteristics were very difficult to differentiate, but even under this handicap, the final results checked well with the proof time and volume scores.

TABLE VIII  
AVERAGE OF 10 DAYS' GASSING-POWER RESULTS ADDING SUCROSE THE FIFTH HOUR<sup>1</sup>

Yeast A		Yeast B		Yeast C		Yeast D	
Total	Hourly	Total	Hourly	Total	Hourly	Total	Hourly
<i>Millimeters of mercury</i>							
107	107	90	90	87	87	102	102
255	148	247	157	224	137	272	170
361	106	361	114	336	112	371	99
403	42	399	38	386	50	407	36
425	22	422	23	413	27	431	24
PRESSUREMETER OPENED AND 0.6156 G. OF SUCROSE STIRRED IN							
141	141	136	136	130	130	140	140
259	118	259	123	242	112	266	126
380	121	372	113	345	103	377	111

<sup>1</sup> Ten g. flour, 0.3 g. of each yeast, and 10 cc. distilled water to start pressuremeter. At fifth hour 0.6156 g. sucrose was stirred in and the pressuremeter returned to water bath.

TABLE IX  
AVERAGE OF 10 DAYS' GASSING-POWER RESULTS ADDING MALTOSE THE FIFTH HOUR<sup>1</sup>

Yeast A		Yeast B		Yeast C		Yeast D	
Total	Hourly	Total	Hourly	Total	Hourly	Total	Hourly
<i>Millimeters of mercury</i>							
103	103	90	90	86	86	111	111
250	147	240	150	221	135	269	158
361	111	358	118	338	117	361	92
396	35	396	38	392	54	400	39
420	24	419	23	417	25	428	28
PRESSUREMETER OPENED AND 0.6156 G. OF MALTOSE STIRRED IN							
127	127	138	138	125	125	147	147
234	107	256	118	215	90	273	126
329	95	355	99	288	73	375	102

<sup>1</sup> Ten g. flour, 0.3 g. of each yeast, and 10 cc. distilled water to start pressuremeter. At fifth hour 0.6156 g. maltose was stirred in and the pressuremeter returned to water bath.

The foregoing procedure was also followed with maltose instead of sucrose at the fifth hour. Average results for 10 days with maltose appear in Table IX. Here again, as in the straight-dough determinations, yeast A did not seem to handle maltose quite as well the first hour, and yeast C showed the best ability to utilize both sugars. A difference occurred here, in that yeast D showed ability to utilize maltose the first hour it was added in the sponge procedure, whereas

this same yeast did not show this utilization when maltose was used in the straight-dough procedure. This may be due to the fact that the induction period for the fermentation of maltose was covered by the sponge fermentation. No particular advantage in using maltose was apparent from these experiments and, since sucrose is used in doughs at this stage in baking, it seems advisable to use sucrose in the evaluation of yeasts. It should be noted, however, that when checking for daily variations in shipments of yeasts, yeasts *A* and *B* showed much wider variations when maltose was used.

### Sponge Pressuremeter Values and Bakes

In running pressuremeter determinations on the yeasts using the sponge method, the same temperature was used in the water bath as was used for the straight doughs (86°F.). The pressuremeter was started with 10 g. of the same flour used for the straight-dough tests, 0.3 g. of each yeast, and 10 cc. distilled water. The pressuremeter was read each hour. At the end of the fifth hour it was removed from the water bath, the lid removed and 0.6156 g. of sucrose stirred in, the lid replaced, and the pressuremeter returned to the water bath. The averages for a 10 day run by this sponge method appear in Table VIII. These data show that yeast *A* and yeast *D* pick up rapidly after the sugar is added, yeast *B* and yeast *C* are slower the first hour after which yeast *B* steps up with yeasts *A* and *D*. Since the critical time for sponge doughs is usually the first hour after the sugar is added, yeasts *A* and *D* should be superior for sponge doughs since they are producing gas at a more rapid rate during this critical time. Yeasts *C* and *D* showed wider daily variations when sucrose was used. Therefore, in checking yeasts for uniformity a technician should try out both sugars over a period of time to learn which will give him the most useful information.

A method of baking a sponge was patterned after the work of Shellenberger and Ziemke (1939). Total weight of flour was 200 g. on a 15% moisture basis. Both the sponge and dough were mixed two minutes in the Swanson G-R mixer. The dough was fermented 20 minutes, divided into two equal portions, rounded up, allowed to rest 15 minutes, panned, proofed to height and baked in the usual manner (425°F.).

	<i>Sponge</i>
Flour	140 g. (undistated)
Yeast	6 g.
Sugar	6 g.
Shortening	4 cc. (liquid)
Water to proper consistency	

	<i>Dough</i>
Flour	60 g.
Sugar	6 g.
Salt	3½ g.
Water to proper consistency	

Sponge time, 5 hours

Exactly the same ingredients were used in the sponge doughs as were used in the straight doughs and the doughs were fermented in the same cabinet at 86°F. This was done with the sponge because no attempt had been made to adjust the water-bath temperature of the sponge pressuremeter determinations. It should be noted that had the temperature of the sponge been lowered to a point more in line with common bake-shop practice, different evaluations of the yeasts might have occurred. This statement is made because, on one set of bakes not included in this report, the fermentation cabinet thermostat stuck and the temperature had been only 79°F. during the sponge fermentation. This bake was continued and the cabinet temperature raised during the mixing, fermentation, rest, and pan proof. Yeast *C*, usually slow proofing, was faster under these conditions and moved up to second place in rate of proof at this lower temperature. When the doughs were ready for the oven, the cabinet temperature had risen to about 84°F.

TABLE X  
DAILY PROOF TIME OF THE FOUR COMMERCIAL YEASTS USING THE SPONGE  
PROCEDURE<sup>1</sup>

First to the oven	Second to the oven	Third to the oven	Fourth to the oven
Yeast <i>D</i> 34½ min.	Yeast <i>A</i> 39½ min.	Yeast <i>B</i> 40½ min.	Yeast <i>C</i> 41 min.
<i>A</i> 36	<i>D</i> 36½	<i>B</i> 39½	<i>C</i> 45
<i>A</i> 38	<i>B</i> 39	<i>D</i> 40	<i>C</i> 43½
<i>D</i> 41	<i>A</i> 42	<i>B</i> 43½	<i>C</i> 44½
<i>B</i> 44½	<i>A</i> 46	<i>D</i> 46½	<i>C</i> 49
<i>A</i> 40½	<i>D</i> 41	<i>B</i> 42½	<i>C</i> 48½
<i>A</i> 42	<i>D</i> 43	<i>B</i> 45	<i>C</i> 46

<sup>1</sup> Averages: yeast *D* 40.2 min., yeast *A* 40.5 min., yeast *B* 42.3 min., yeast *C* 45.3 min.

The averages for seven days' baking for proof time (proofing to height) appear in Table X. Yeasts *A* and *D* proofed up according to the pressuremeter determinations (gas produced the first hour after sugar added). Yeasts *C* and *D* showed the widest range of proofing time over the seven-day bake period. Even this was in line with the pressuremeter, for yeasts *C* and *D* showed the widest daily variation when sucrose was used. In bake-shop conditions where both sugars are available, perhaps this difference would level off, for yeasts *A* and *B* showed wider variations when maltose alone was used in the pressuremeter determinations.

Table XI shows the composite score of the interior and exterior of the loaves. In order to eliminate any tendency to depend on the pressuremeter results in evaluation, one technician baked and numbered the loaves in code, while the other technician graded the pairs.

TABLE XI

COMPOSITE INTERIOR AND EXTERIOR EVALUATION OF DUPLICATE LOAVES USING  
FOUR COMMERCIAL YEASTS IN THE SPONGE BAKING  
PROCEDURE—SEVEN DAYS

First in loaf quality	Second in loaf quality	Third in loaf quality	Fourth in loaf quality
Yeast <i>A</i>	Yeast <i>D</i>	Yeast <i>C</i>	Yeast <i>B</i>
<i>A</i>	<i>B</i>	<i>D</i>	<i>C</i>
<i>A</i>	<i>B</i>	<i>D</i>	<i>C</i>
<i>D</i>	<i>A</i>	<i>B</i>	<i>C</i>
<i>A</i>	<i>B</i>	<i>C</i>	<i>D</i>
<i>A</i>	<i>D</i>	<i>B</i>	<i>C</i>
<i>B</i>	<i>A</i>	<i>C</i>	<i>D</i>

Here again the technicians alternated in baking and in grading. Yeast *A* was graded first consistently. Yeast *D*, however, did not stay in line with the pressuremeter, for yeast *B* excelled yeast *D* in loaf quality. Yeast *C* was last as indicated by the pressuremeter. The fact that yeasts *B* and *D* changed places here may be due to differences in the way the two yeasts affect the gas-retention properties of the dough.

TABLE XII

VOLUMES OBTAINED DURING THE SEVEN DAYS' BAKE USING FOUR COMMERCIAL  
YEASTS IN THE SPONGE BAKING PROCEDURE<sup>1</sup>

First in volume	Second in volume	Third in volume	Fourth in volume
Yeast <i>D</i> 800 cc.	Yeast <i>A</i> 740 cc.	Yeast <i>B</i> 735 cc.	Yeast <i>C</i> 705 cc.
<i>B</i> 700	<i>A</i> 695	<i>D</i> 690	<i>C</i> 680
<i>A</i> 720	<i>B</i> 710	<i>D</i> 698	<i>C</i> 688
<i>B</i> 705	<i>A</i> 690	<i>D</i> 680	<i>C</i> 600
<i>A</i> 720	<i>D</i> 705	<i>C</i> 700	<i>B</i> 670
<i>A</i> 695	<i>D</i> 690	<i>B</i> 685	<i>C</i> 625
<i>D</i> 700	<i>A</i> 690	<i>B</i> 680	<i>C</i> 650

<sup>1</sup> Average loaf volume: yeast *D* 707.5 cc., yeast *A* 707 cc., yeast *B* 695 cc., and yeast *C* 664 cc.

In Table XII appear the volume scores of the loaves in the seven bakes. It may be observed that yeast *D* has the highest loaf volume of any of the yeasts and yeast *B* is third in volume. Using the 3-2-1

TABLE XIII

COMBINED DATA OF PRESSUREMETER, PROOF-TIME, LOAF-VOLUME, AND  
COMPOSITE SCORE—SPONGE-DOUGH PROCEDURE

Yeast	Average sixth-hour gas production	Average proof time	Average loaf volume	Average composite score
<i>A</i>	141 mm.	40.5 min.	707 cc.	First
<i>B</i>	136	42.3	695	Second
<i>C</i>	130	45.3	664	Fourth
<i>D</i>	140	40.2	707½	Third



system in scoring, we find that yeasts *A* and *D* have exactly the same score on volume.

Table XIII combines all these data on the sponge pressuremeter and sponge baking. The millimeters of pressure in the pressuremeter the first hour after the sugar is introduced are a very good indication of how that yeast will behave in a sponge baking procedure.

### Summary and Conclusions

The pressuremeter was used on an unmalted flour to determine the characteristics and uniformity of various commercial yeasts. The data obtained with the pressuremeter under various conditions correlated very well with data obtained by actual test baking both on straight-dough and sponge methods. The pressuremeter shows very slight variations in daily shipments of yeast that cannot be found in the bake and for this reason slight daily variations could well be discounted. The pressuremeter shows very marked differences between various commercial yeasts, and by proper manipulation it will show certain yeasts more adaptable to certain conditions. If the conditions under which a yeast is to be used are known, the pressuremeter will assist in determining yeast characteristics that will best be suited to this set of conditions.

The pressuremeter equipment of Sandstedt and Blish has a definite place in the cereal laboratory for testing yeast uniformity and character. It will differentiate yeasts in a manner that will in most cases be correlated with the baking test.

### Literature Cited

- Bailey, C. H., and Johnson, A. H.  
1924 Carbon dioxide diffusion ratio of wheat flour doughs as a measure of fermentation period. *Cereal Chem.* 1: 293-304.
- Blish, M. J., Sandstedt, R. M., and Astleford, G. R.  
1932 Sugars, diastatic activity and gassing power in flours. *Cereal Chem.* 9: 378-393.
- Bohn, R. T., and Favor, H. H.  
1938 Evaluation of yeast activity. *Cereal Chem.* 16: 238-252.
- Brabender, C. W.  
1934 Six years farinography. *Cereal Chem.* 11: 586-597.
- Cook, W. H., and Malloch, J. G.  
1930 Yeast testing. *Cereal Chem.* 7: 133-142.
- Sandstedt, R. M., and Blish, M. J.  
1934 Yeast variability and its control in flour gassing power tests. *Cereal Chem.* 11: 368-383.
- Shellenberger, J. A., and Ziemke, W. H.  
1939 A critical study of a "pup" sponge baking method. *Cereal Chem.* 16: 32-35.
- Sullivan, B., and Near, C.  
1935 A study of yeast variability as measured by the fermentograph. *Cereal Chem.* 12: 506-511.

## **EFFECT OF STORAGE TEMPERATURES UPON THE VIABILITY AND BAKING PROPERTIES OF COMPRESSED YEAST**

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(Read at the Annual Meeting, May 1939)

It is not always possible to have freshly produced compressed yeast delivered to the bakery daily. Hence it becomes necessary to store the yeast for a short or perhaps even a long period of time before use. The question then arises as to what temperature should be selected for storing the yeast. Should the yeast be frozen? If so, what procedure should be employed in getting the yeast in condition for baking?

Cook and Malloch (1930) state that yeast stored at about 32°F. up to ten days of time lost very little in gas production. Harrel (1926) stored yeast at 50°, 80°, 90°, and 100°F. and at the end of 24 hours he determined gas strength and made baking tests. His results showed that with yeasts stored for the same length of time there was a steady decrease in the gas-producing power, as the temperature was increased. Staiger and Glaubitz (1929) on the other hand stored yeast at low temperatures, even below freezing. They found that yeast which was frozen at from +14°F. to -13°F. for one to four days and subsequently thawed at room temperature and at 41°F. respectively, showed but little change in properties. The baking and keeping qualities of the yeast as well as the nitrogen content and biological appearance were almost normal.

Iwanowski and Brezezinski (1934) studied the effect of time upon yeast stored at different temperatures. They found that yeast could be stored at 32°F. for two to three months without marked deterioration and at 56°F. for about two weeks, after which time the yeast would have about 10% to 20% dead cells; and that after storage at 72°F. for one week it would have approximately 20% of dead cells. Their conclusion was that the yeast could be stored at these temperatures and for these periods of time without marked changes in its physiological state, except ability to bud, and without losing commercial value.

Weaver, Talbott, and Coleman (1933) in testing yeast variability studied two brands of yeasts. Their test consisted of baking 50 replicate loaves using each brand of yeast and taking the mean loaf volume as a criterion of change. They held the yeast at room temperature for

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<sup>1</sup> Bureau of Chemistry and Soils.

<sup>2</sup> Food and Drug Administration.

seven hours on each baking day and at an electrical refrigerator temperature the rest of the time. Baking tests were made with the fresh yeast six hours old and again after 24, 48, 96, and 168 hours; their results showed that with one of the brands of yeast there was practically no change up to 96 hours and with the other one none even up to 168 hours.

In order to gather further information on this storage problem, a study was made as detailed herewith. Five pounds of compressed yeast packed in crumpled newspapers and placed in a pasteboard carton along with dry ice were transported by airplane across the continent from the Pacific coast. The yeast was quite cold but not frozen at the time of arrival. Baking tests were made immediately with this yeast in comparison with fresh, unfrozen yeast of another brand, after which one-pound cakes of both brands of yeast were placed in storage at 0°, 20°, 30°, and 45°F. At the end of one month, two months, and three months, respectively, portions of the cakes of yeasts were removed from storage and tested for plate counts of yeast cells, percentage of dead cells, pH determinations, and baking quality. Two methods were used in thawing the yeast preparatory to baking. In one method the frozen yeast was suspended in 50 cc. of ice water and placed at once into the dough mix, with the balance of the water warm enough to yield a dough of proper temperature; in the other method the yeast was removed from the freezing room to a 50°F. room, allowed to thaw overnight, and then used in the usual manner. This same procedure was used throughout all the tests.

The first test was made upon receipt of samples.

TABLE I

COMPARISON OF FRESH YEAST WITH YEAST TRANSPORTED ACROSS THE CONTINENT BY AIRPLANE WITH DRY-ICE REFRIGERATION

	Yeast A, transported sample	Yeast B, fresh sample
Plate count ( <i>million cells per g.</i> )	1,020	3,000
Dead cells, %	2	1
pH of yeast	5.5	5.6
Loaf volume, cc.	2,290	2,250

The transported-sample yeast (*A*) was somewhat more yellow in color than the fresh-sample yeast (*B*). Yeast *A* was slightly soft and sticky when received. It made a very good loaf of bread, almost the same kind of loaf as did yeast *B*.

The odd-numbered loaves (Figure 1) were made with yeast *B* and the even-numbered loaves with yeast *A*, with one exception. The loaves

TABLE II  
RESULTS OF TESTS MADE WITH THE YEASTS AFTER ONE MONTH'S STORAGE

	0° F.—Yeast		20° F.—Yeast		30° F.—Yeast		45° F.—Yeast		0° F.—20° F.		
	B	A	B	A	B	A	B	A	Yeast A		
THAWED IN ICE WATER											
No. in figure	1	2	3	4	5	6	7	8	15		
Cell count ( <i>million per g.</i> )	4,300	820	1,560	1,050	4,800	2,400	7,920	1,440	130		
Dead cells, %	0	20	4	16	0	4	0	10	8		
pH of yeast	5.2	5.9	4.7	5.5	4.7	6.9	6.7	7.6	5.4		
Loaf vol., cc. <sup>1</sup>	1,950	1,790	2,200	2,075	2,300	2,200	2,030	1,875	21"×11½"		
Loaf character	Good	Poor	Very good	Fair	Very good	Very good	Good	Fair	Poor		
THAWED OVERNIGHT BEFORE USING											
No. in figure	9	10	11	12	13	14	16				
Cell count ( <i>million per g.</i> )	4,400	1,600	6,000	1,680	4,320	2,100	410				
Dead cells, %	4	16	0	6	0	0	30				
pH of yeast	5.3	5.6	5.3	5.6	4.9	6.8	5.6				
Loaf vol., cc.	2,100	1,800	2,075	2,050	2,180	2,000	20½"×12"				
Loaf character	Good	Poor	Good	Good	Good	Good	Poor				

<sup>1</sup> When the loaf was too small to measure in the loaf-volume apparatus the longitudinal and transverse circumferences, in inches, were measured.

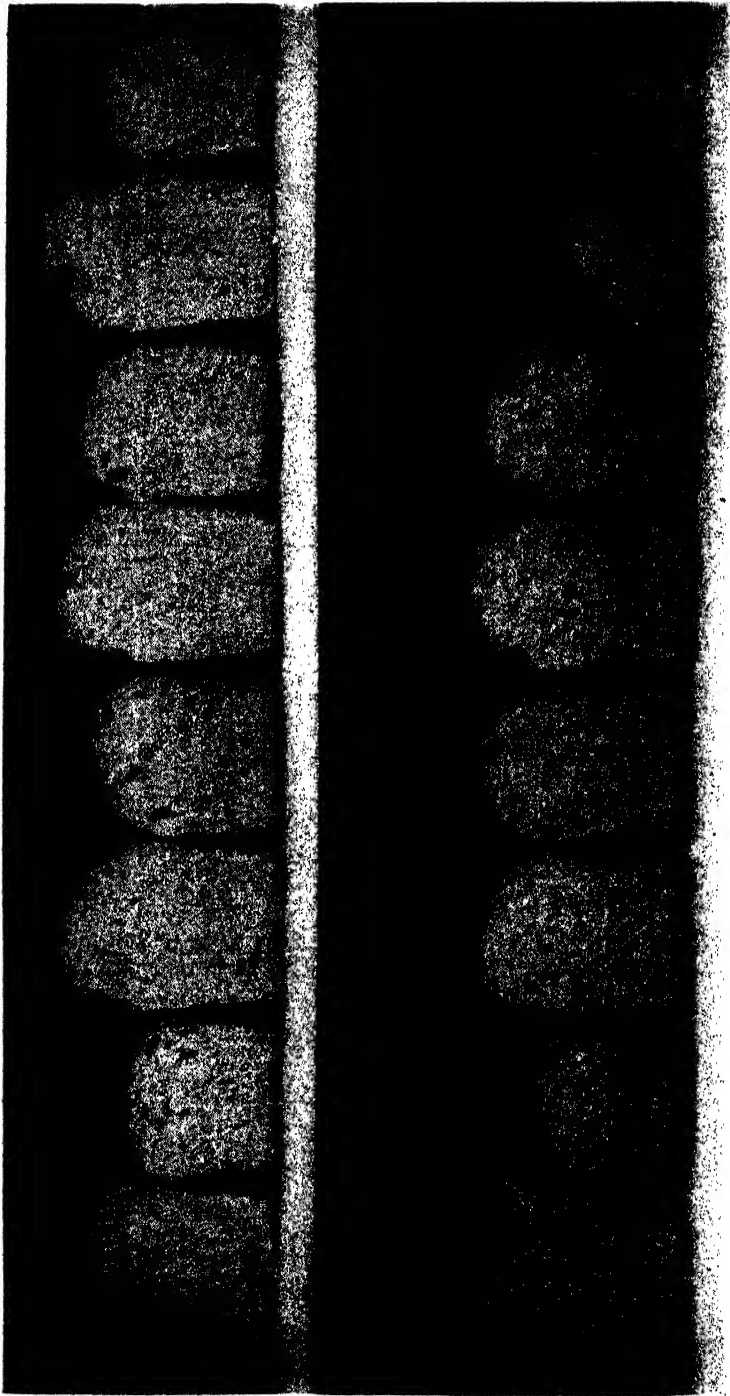


Fig. 1. One month's storage of yeast. A, transported yeast packed in dry ice; B, fresh yeast.

Loaf No.	Yeast	Storage temp., °F.	Loaf No.	Yeast	Storage temp., °F.
1	B	0	9	B	0
2	A	0	10	A	0
3	B	20	11	B	20
4	A	20	12	A	20
5	B	30	13	B	30
6	A	30	14	A	30
7	B	45	15	A	0
8	A	45	16	A	0

Suspended in ice water, used at once.

Thawed at 50° F. overnight, then used.

For 6 days, then at 20° F. 27 days, then used.

For 6 days, then at 20° F. 27 days, thawed overnight.

on the top row were made by suspending the frozen yeast in ice water and then incorporating it in the doughs immediately, while the corresponding six loaves on the left of the picture in the bottom row were made from yeast that had thawed overnight at 50°F. and then was incorporated in the doughs.

The first pair of loaves toward the left on both top and bottom rows were made with yeast stored at 0°F., the second pair with yeast stored at 20°F., the third at 30°F., and the last pair on the top row were made from yeast stored at 45°F. On the bottom row the last two loaves to the right were made with yeast *A*, which had been stored at 0°F. for 6 days and then at 20°F. for 27 days. Loaf 15 was made with frozen yeast suspended in ice water and used at once, while for loaf 16 the yeast was thawed overnight in the 50°F. room and then used. This cake of yeast *A* had greatly deteriorated by the end of the first month's storage.

As shown in Figure 1, there had been some deterioration of the yeast *A* after one month's storage. This was especially noticeable where the yeast had been stored at 0°F. and at 45°F. and also with the yeast that at first was stored at 0° for six days and then transferred to 20°F. for 27 days (15 and 16 in Figure 1).

After two months' storage the deterioration was still more pronounced (as shown in Figure 2). By this time there were no satisfactory loaves made from yeast *A* stored at any of the different temperatures. Yeast *B* still made quite good loaves, when stored at all four temperatures. Yeast *B* had not been packed with dry ice; hence it had not been subjected to such extreme cold as had yeast *A*.

After three months' storage, the deterioration of the yeast was still more pronounced (as shown in Fig. 3). At this time none of the loaves was entirely satisfactory. Even though some of the loaves made with yeast *B* had good external appearance, the crumb was inferior. The grain was coarse and texture firm. All the loaves made from yeast *A* were quite unsatisfactory.

The yeasts stored at 0°F. did not make quite as good loaves as those stored at 20° or 30°F. At 45°F. the yeast darkened in color and crumbled badly. This yeast did not keep as well as that stored at 20° or 30°F., but when not previously frozen produced satisfactory results up to the end of two months' storage.

All of the yeast being tested had incorporated with it some starch, and upon freezing these starch cells ruptured and the result was that when the frozen yeast was thawed it softened down into a pasty mass. This made the process of handling more difficult.

At 30°F. the yeast did not freeze and hence did not lose its normal physical condition, and yet it continued to produce just as satisfactory bread as yeast stored at any other temperature.

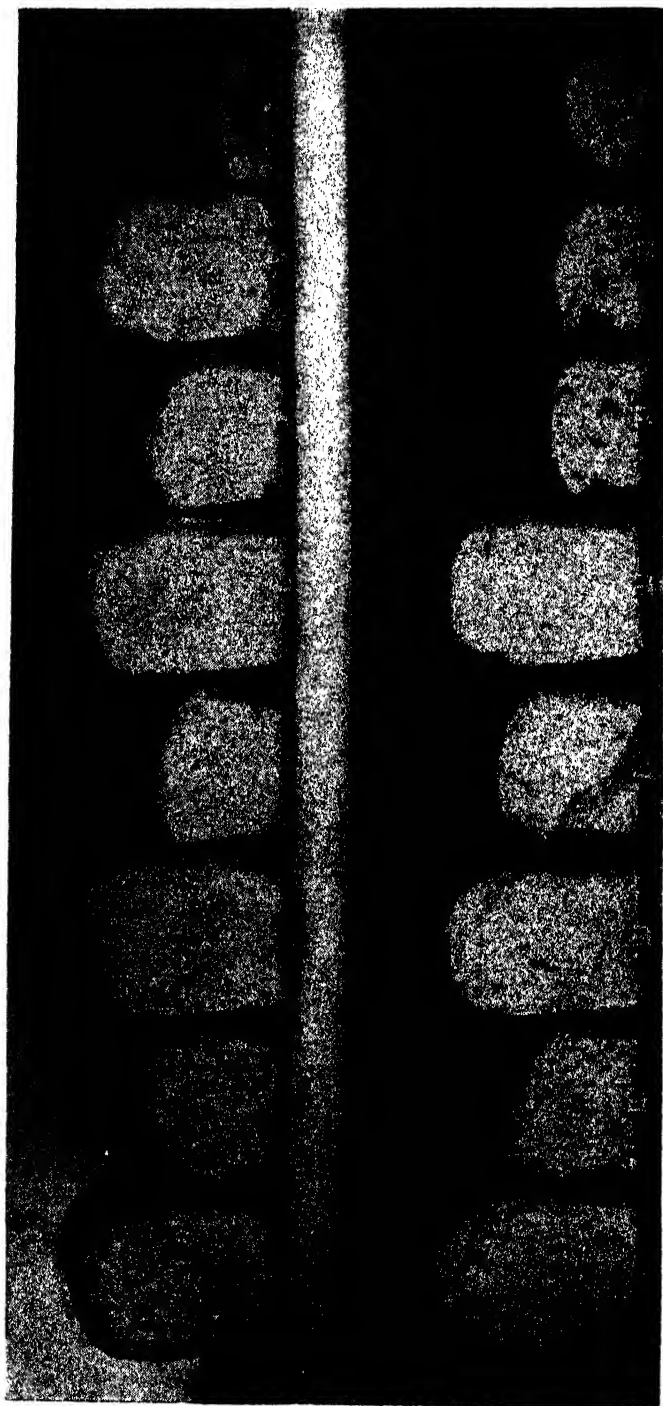


Fig. 2. After two months' storage of yeast. (Loaves correspond to those in Fig. 1, with the exception that loaves 15 and 16 were stored at 20° F. for 56 days.)

TABLE III  
RESULTS OF TESTS MADE WITH THE YEASTS AFTER TWO MONTHS' STORAGE

0° F.—Yeast		20° F.—Yeast		30° F.—Yeast		45° F.—Yeast		0° F.—20° F.
B	A	B	A	B	A	B	A	Yeast A
THAWED IN ICE WATER								
No. in figure	1	2	3	4	5	6	7	8
Cell count (million per g.)	9,000	1,080	2,700	750	2,000	240	5,600	Less than 1
Dead cells, %	7	20	3	30	0	2	1	85
pH of yeast	4.8	5.4	4.7	5.0	5.5	7.3	7.8	19 1/4" × 6"
Loaf vol. <sup>1</sup>	2,230	21" × 12"	2,275	22" × 12 1/4"	2,300	21 1/4" × 12 1/4"	2,150	19 1/4" × 10 1/4"
Loaf character	Good	Small, poor	Good	Fair	Very good	Small, poor	Good	Very small, very poor
THAWED OVERNIGHT BEFORE USING								
No. in figure	9	10	11	12	13	14	15	16
Cell count (million per g.)	210	960	2,100	760	3,200	1,160	300	300
Dead cells, %	8	36	6	9	0	7	90	90
pH of yeast	5.4	5.6	4.9	5.5	5.8	7.3	5.4	19 1/4" × 9 1/4"
Loaf vol.	2,230	21 3/4" × 12"	2,125	23" × 13"	2,275	19 1/4" × 10 3/8"	19 1/4" × 9 1/4"	Small, poor
Loaf character	Very good	Small, poor	Good	Fair	Very good	Small, very poor	—	—

<sup>1</sup> See footnote 1, Table II.



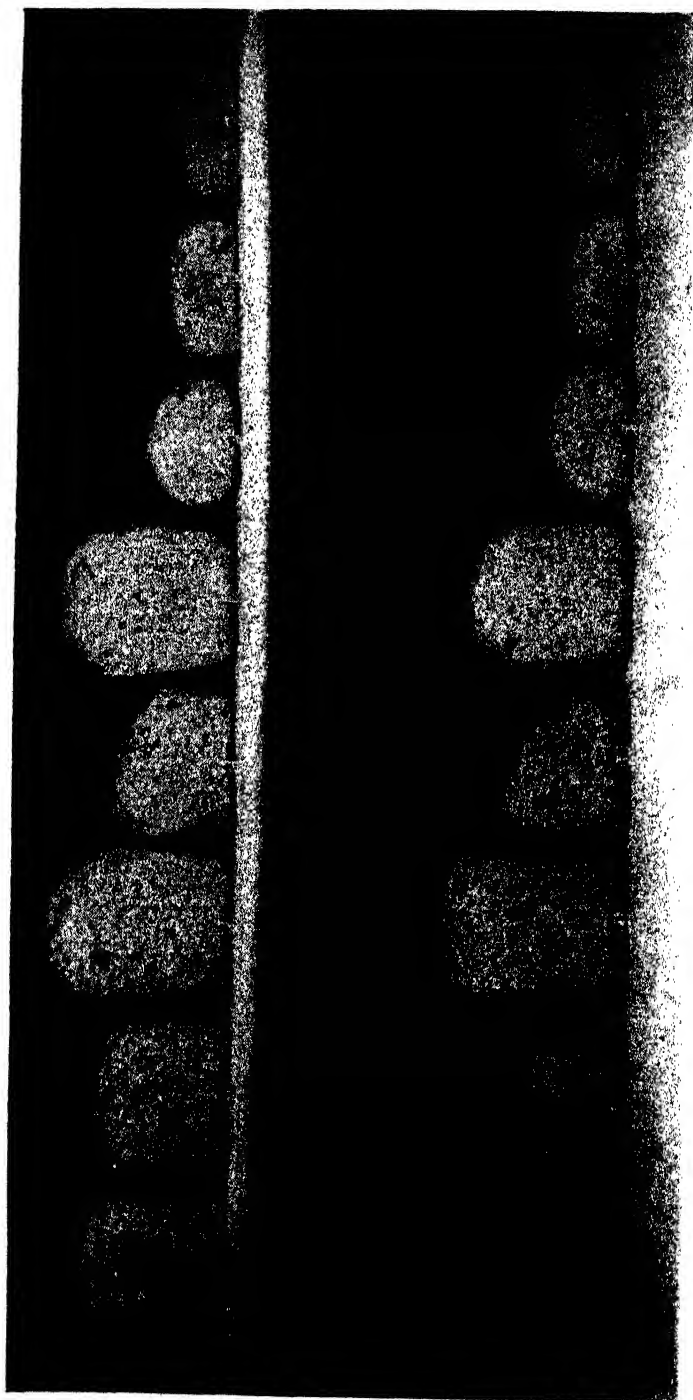


Fig. 3. After three months' storage of yeast. (Loaves correspond to those in Figs. 1 and 2, with the exception the 15 and 16 were stored at 20° F. for 89 days.)

TABLE IV  
RESULTS OF TESTS MADE WITH YEASTS AFTER THREE MONTHS' STORAGE

0° F.—Yeast		20° F.—Yeast		30° F.—Yeast		45° F.—Yeast		0° F.—20° F.
B	A	B	A	B	A	B	A	Yeast A
THAWED IN ICE WATER								
No. in figure	1	2	3	4	5	6	7	8
Cell count ( <i>million per g.</i> )	120,000	3,100	9,700	2,400	3,700	160	20	7/100
Dead cells, %	15	15	11	45	0	25	80	95
pH of yeast	4.7	5.4	4.4	5.1	5.9	7.3	7.8	7.0
Loaf vol. <sup>1</sup>	24"×13½"	23¼"×13¼"	25"×15½"	20½"×11½"	24½"×14½"	19½"×9"	18½"×8½"	18½"×8½"
	1,875	1,750	2,150	—	2,025	—	—	—
Loaf character	Fair	Poor	Good	Poor	Good	Very poor	Very poor	Very poor
THAWED OVERNIGHT BEFORE USING								
No. in figure	9	10	11	12	13	14	—	16
Cell count ( <i>million per g.</i> )	4,500	2,000	6,300	2,600	10,800	320	—	1,600
Dead cells, %	16	35	12	32	1	3.4	—	96
pH of yeast	5.4	5.6	4.3	5.2	6.6	7.2	—	5.5
Loaf vol.	26¼"×16¼"	21¼"×11½"	25"×15½"	21½"×11½"	23½"×13½"	19½"×9½"	—	19"×8½"
	2,500	—	2,170	—	1,850	—	—	—
Loaf character	Good	Very poor	Medium	Very poor	Fair	Very poor	—	Very poor

<sup>1</sup> See footnote 1, Table II.

While frozen yeast can be suspended in ice water and used immediately, the balance of the water must be sufficiently warm to offset this low temperature so that normal fermentation can proceed; consequently preference is given to the method of allowing the yeast to thaw slowly at approximately 50°F. overnight, and then incorporating it in the dough in the usual manner.

Since yeast *A* made very good bread when it was first received but deteriorated more rapidly upon storage than did yeast *B*, it was thought that perhaps the low temperature to which yeast *A* had been subjected when it was packed in dry ice for shipment might have been the cause of the more rapid deterioration. In order to test this possibility, two one-pound packages of yeast were obtained. One of them was surrounded with crumpled paper in a pasteboard carton and dry ice placed in the container over the cake of yeast. Upon examination the following day, it was observed that the dry ice had evaporated and more was added. At the end of 48 hours the carton was placed in a room at 20°F. and kept at that temperature for one month.

The second cake of yeast was handled in the same manner except that it was placed in a vacuum-walled jar similar to a large thermos bottle, with the dry ice immediately surrounding the wrapper on the cake of yeast. After two days of this treatment the yeast was transferred to a pasteboard carton and placed in a room at 0°F. for one month.



Fig. 4. Comparison of fresh and frozen yeast.

Fig. 5. Comparison of fresh yeast with yeast frozen at -109° F. for 24 hours

After the month's storage these two yeasts were compared with fresh yeast in making bread. The result is shown in Figure 4. It will be seen that great injury had been done to the yeast. Loaf 2 was made with the yeast that was packed in the pasteboard carton along with the dry ice and subsequently stored at 20°F. This treatment was intended to simulate that given yeast *A*.

Loaf 3 was made with the yeast that had been given the more severe treatment, but the loaf was only slightly inferior to loaf 2.

The microscopical examination of these yeasts showed both by cell count and percentage of dead cells that one month's storage after sharp freezing for two days was detrimental to the yeast and that a temperature of 0°F. was more harmful than 20°F.

In order to determine what proportion of this damage was due to subjecting the yeast to the low temperature and what proportion to the storage period, another sample of yeast was placed in the vacuum-walled jar with a goodly portion of dry ice and the temperature surrounding the yeast was determined to be that of evaporating dry ice, -109°F. After 24 hours at that temperature the yeast was transferred to an electrical refrigerator for another 24 hours; it was then used in baking in comparison with fresh yeast that had not been subjected to a low temperature. The result may be seen in Figure 5. While the external appearance of this loaf 2 was almost as good as that of loaf 1 made with the fresh yeast, the internal characteristics were not nearly so good. The grain was coarse, with thick cell walls, the texture was more firm, and the color was slightly darker. The crust color also showed more caramelization. It is interesting to note that a living organism like yeast can withstand such extremely low temperature for 24 hours and later resume biological activity. These latter tests indicate that some damage is done the yeast when it freezes and that this damage is more pronounced as the subsequent storage period is prolonged.

### Summary

Compressed yeast packed with dry ice for a short period (two days) was stored along with fresh compressed yeast at 0°, 20°, 30°, and 45°F. for three months. Microscopical, pH, and baking tests were made at the beginning and at the end of one month's, two months', and three months' storage, respectively, at the temperatures indicated above.

The yeast that had been packed with dry ice deteriorated more rapidly than the fresh yeast stored for the same length of time and at the same temperatures. The frozen yeast became mushy after it was thawed, making it more difficult to handle.

Of the different storage temperatures used, 30°F. is considered the most suitable since the yeast did not freeze at that temperature, nor lose its normal consistency, and bread made from this yeast was fully equal to that made with yeast stored at any other temperature.

The temperature of evaporating dry ice (-109°F.) injures yeast in 24 hours and subsequent storage below freezing increases the amount of deterioration.

The general conclusion to be drawn as a result of this investigation is that approximately 30°F. is the most suitable temperature for the storage of compressed yeast.

**Literature Cited**

- Cook, W. H., and Malloch, J. G.  
1930 Yeast testing. *Cereal Chem.* 7: 133-142.
- Harrel, C. G.  
1926 Some variable factors of bread production. *Cereal Chem.* 3: 1-18.
- Iwanowski, W., and Brezezinski, K.  
1934 Changes in pressed yeast during storage (translated title). *Przemysl Chem.* 18: 93-101.
- Staiger and Glaubitz  
1929 Frozen yeast (translated title). *Z. Spiritsusind.* 52: 116.
- Weaver, Ray, Talbott, Philip, and Coleman, D. A.  
1933 Variability in experimental baking. II. Yeast variability. *Cereal Chem.* 10: 617-618.

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**LABORATORY MALTING. III. STEEPING EQUIPMENT  
AND METHOD<sup>1</sup>**

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(Read at the Annual Meeting, May 1939)

The first paper in this series described equipment for making batches of malts in the laboratory under reproducible conditions and on a routine scale. The germinators and kilns have proved reasonably satisfactory but the steep tank required modification. After considerable experimentation steeping equipment of an entirely different type was designed and constructed. It has now been in operation for some time and since it has proved generally satisfactory and contains certain advantageous automatic features, it appeared worth while to publish a description of it.

It is the practice in our laboratories, as in the Malting Laboratory at the University of Wisconsin (Dickson, Shands, Dickson, and Burkhardt, 1935), to steep all samples to the same moisture content. Owing to differences in kernel size and certain varietal effects (cf. Meredith and Anderson, 1938) samples differ considerably in the time they take to reach the desired moisture content, 44% in our laboratories. The time required by each sample is determined in practice by means of pilot steeping experiments which can readily be carried out under routine conditions.

In making a batch of malts all samples must be removed from the steep at the same time. Thus, since they may require different lengths of steep, it will be obvious that they may have to be put into the steep at different times. In order to have a fully automatic steep tank, it is

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<sup>1</sup> Published as paper No. 167 of the Associate Committee on Grain Research of the National Research Council of Canada and the Dominion Department of Agriculture, and as N. R. C. No. 858.

therefore necessary to arrange not only for automatic changing of water and automatic aeration, but also for an automatic method for starting the steeping of each individual sample at any predetermined hour during the day or night.

Such equipment has been devised and models of it have been installed both in the National Research Laboratories and at the University of Manitoba. It has increased the precision of the malting

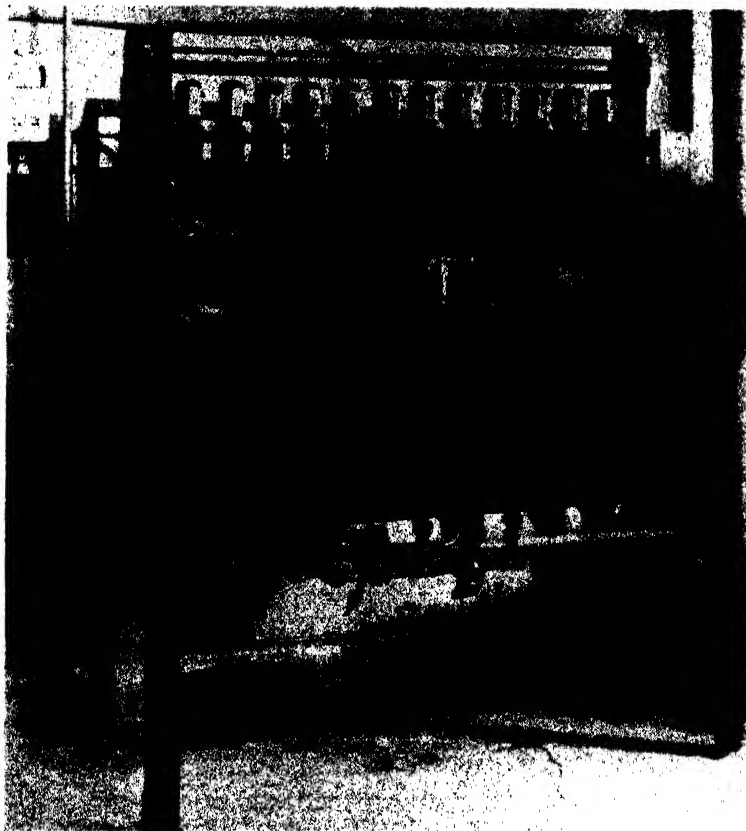


Fig. 1. Steep tank.

test and has also made it unnecessary for members of the malting staff to visit the laboratories outside of working hours for the purpose of attending to steeps.

The first of these new steep tanks was designed in Ottawa and constructed partly from pieces of the old equipment. When it had been shown that it operated satisfactorily, an improved model was designed, built by a Winnipeg firm, and installed at the University of Manitoba. It is this model that is described in this paper.

### Description of Equipment

The equipment consists essentially of a long narrow steep tank standing inside a larger insulated water tank. The inner tank is connected to the large tank and to waste by pipes fitted with solenoid valves operated by an electric time switch. This mechanism permits the inner tank to be drained and refilled automatically at intervals, thus providing changes of water and periods of aeration for the samples in the inner steep tank. A second automatic feature, consisting of a battery of alarm clocks, makes it possible to drop samples into the steep tank at predetermined times.

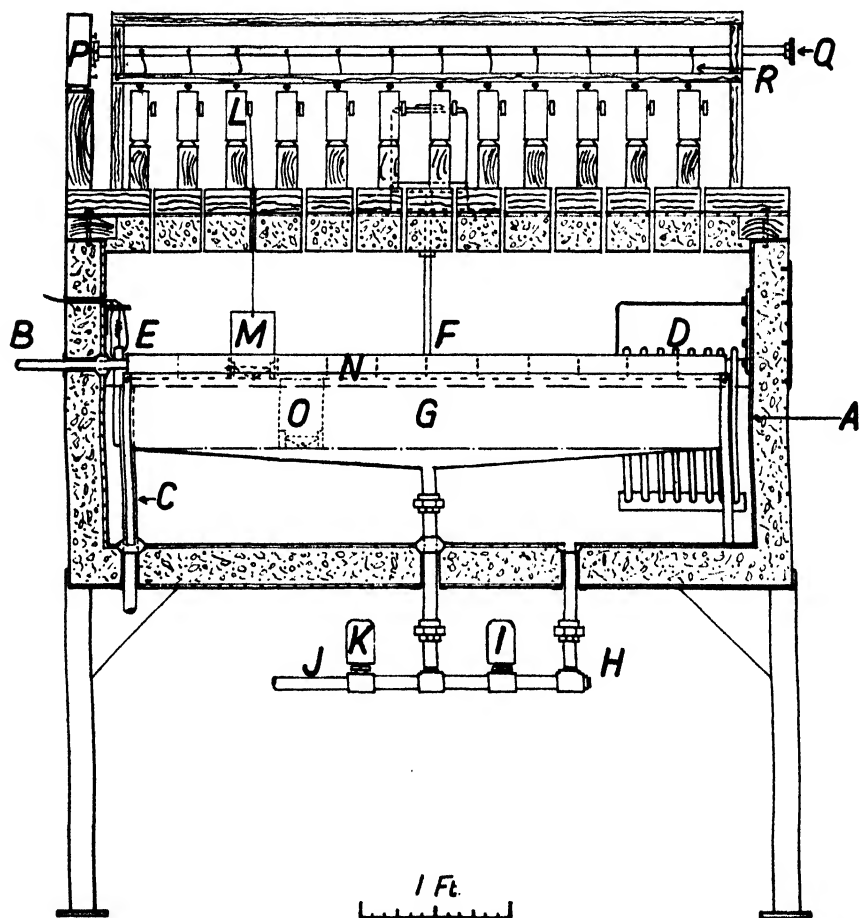


Fig. 2. Front elevation of steep tank.

A photograph of the equipment is shown in Figure 1 and details of its construction are given in Figure 2, which shows the front elevation of the unit in section. The large tank *A* is constructed of  $\frac{3}{16}$ " boiler plate insulated with three inches of cork, and enclosed in an outer casing with  $\frac{5}{16}$ " boiler plate bottom and 18 gauge sheet metal sides.

The cover of the tank is removable and of the same construction with boiler plate top and cork insulation protected by sheet metal. The water level in the tank is kept constant by a float valve attached to the water inlet *B*. There is also a standing waste which is shown at *C*. The flow of refrigerant through the evaporator coil *D* is controlled by the mercury in glass thermoregulator *E*. This is connected to a relay in the live line to the compressor motor (not shown in the drawing), throwing it on and off as required. The water in the tank is circulated by a stirrer shown at *F*.

The inner tank *G* in which the samples are steeped is constructed of 14-gauge galvanized iron. It holds twenty-four 250-g. samples, each in a galvanized iron cage (Fig. 3) with holes in the bottom to allow

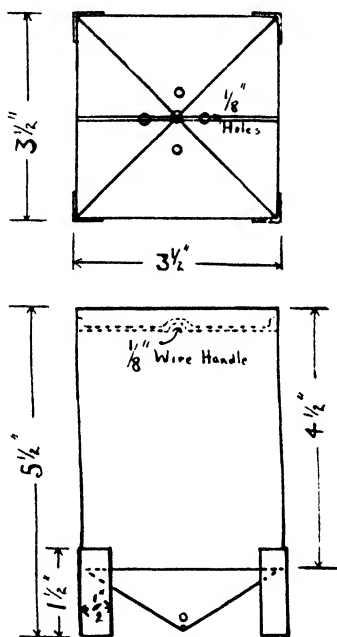


Fig. 3. Steep cage.

entrance of water. These cages rest on a false bottom of wire mesh (6 mesh) during steeping. The inner and outer tanks are connected by the  $\frac{3}{4}$ " pipe *H* in which the water flow is controlled by the solenoid valve *I*. The valve is normally open so that the water in the two tanks stands at the same level. The connections to waste are seen at *J* with a normally closed solenoid valve *K* in the line.

The alarm clocks *L* by which the cages are dropped are mounted on individual wooden blocks, and these blocks are in turn mounted on a 6"  $\times$  2" plank laid on top of the tank cover. The cages are suspended from the alarm winding key of the clocks by piano wire and



curtain rings. A cage is shown suspended from a clock at *M*. The bottom of the cage fits into guides *N* so that when the alarm rings and the alarm key turns the cage drops cleanly into the water as shown at *O*. The alarm clocks have twelve-hour movements, but a twenty-two-hour control is supplied by the master clock *P*. The alarm wind of this clock is attached to a shaft *Q* to which the alarm-release pins of the other clocks are attached by string as shown at *R*.

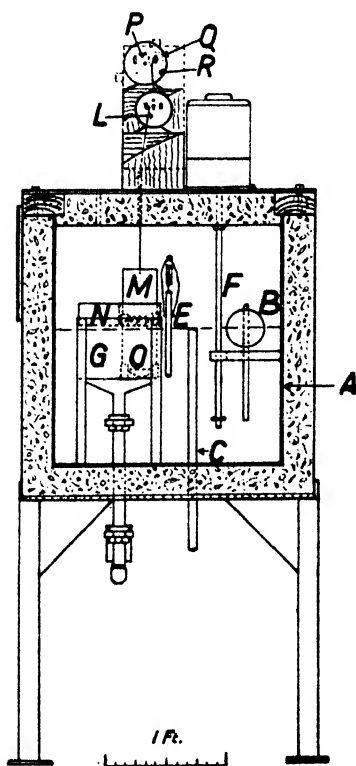


Fig. 4. Side elevation of steep tank.

A side view of the equipment is shown in section in Figure 4. This diagram shows that the inner tank *G* is situated close to the doors in the front portion of the large tank. The space behind the inner tank is occupied by the cooling coil (not shown), stirrer *F*, float valve *B*, standing waste *C*, and thermoregulator *E*.

### Operation of Steep Tank

The steep water is thermostatically controlled at 10°C. and only the water that is drained from the inner tank twice daily is replaced. This draining is done by the action on the solenoid valves of an electric clock with automatic switch gear. The clock is set so that the current is applied to the valves at 8 A.M. and 8 P.M. each day. When this hap-

pens valve *I* closes and shuts off the supply of water to the inner tank, while valve *K* opens allowing the water in the inner tank to flow to waste. The valves are maintained in this position for one hour. At the end of the one-hour aeration period the current is shut off by the clock and the valves return to their normal positions. The valve on the waste outlet closes and the valve on the connecting line between the inner and the outer tank opens. The water in the inner tank rises to the level of that in the outer tank, which is maintained by means of a supply line controlled by a float valve.

The practice of this laboratory (Manitoba) is to malt in batches of twelve, so that only twelve clocks are required for dropping the samples into the steep. Allowance has been made in the inner tank for 24 samples as it is sometimes necessary to start steeping samples in a second batch before the first batch is removed.

In preparing a batch, each sample is suspended from its respective clock, the alarm of which is set to ring at the hour that the sample should go into the steep. If the sample is to drop into the steep within 11 hours the alarm release is taken out. When the alarm rings the winding key revolves and the curtain ring slips off, dropping the cage.

When the sample should go into the steep after 11 hours the master clock mechanism *P* is brought into use. The alarm releases on the clocks have been altered and made completely removable, and they are tied to the shaft *Q* operated by the alarm wind of the master clock. The alarm of the master clock is set to ring 11 hours from the time of preparation, so that the clocks bearing samples to go in after this interval have the alarm releases left in for the first 11 hours and these are subsequently pulled by the master clock. Since clocks do not ring while the alarm release is in, clocks controlled by the master clock do not drop samples until after 11 hours. The clock mechanism thus makes it possible to drop 12 samples into the steep, each at the required time, during the 22 hours after the mechanism is set.

### Removal of Samples from Steep

At the end of the steeping period the cages are drained by hanging them inside the tank for 30 minutes. They are then removed and the few drops of water still adhering to the barley are removed by suction. A cup that fits the bottom of the cages is attached to a suction flask, which in turn is connected to a water pump. The samples are then weighed and transferred to the germination cages (Anderson and Rowland, 1937). Adjustment to the desired moisture content of 44% can be made by adding a few grams of water or by removing a few grams with blotting paper as reported by Sallans and Anderson (1939).

### Precision

The clocks drop the samples into the steep within five minutes of the setting time and have proved entirely satisfactory. Over the six-month period that the equipment has been in use at the University of Manitoba the average amount by which the steeped samples have differed from the required weight of 446 g. is 3.4 g. The standard deviation is 4.5 g., which shows that the error of steeping is only 1.0%. The errors of the Ottawa equipment are of the same order. These errors represent the combination of the error of estimate from the pilot steeps and the actual error of steeping. The equipment therefore possesses a very satisfactory level of precision.

### Summary

Laboratory steeping equipment for twenty-four 250-gram samples is described. The steep tank is built into a larger water tank which is thermostatically controlled at 10°C. The steep tank is connected to the large tank and to waste. Solenoid valves, operated by an electric time switch, permit the steep tank to be automatically emptied and refilled, thus providing aeration and a change of water for the samples.

A second automatic feature, consisting of 13 alarm clocks, permits each individual sample to be dropped into the steep tank at the required time, at any hour of the day or night. The length of time required for each sample to steep to a moisture content of 44% is determined by a pilot steeping test. The combined error of pilot test and steeping is quite small. On the average, samples are within 3.4 g. of the required weight when they are removed from the steep tank. The standard deviation is 4.5 g. and represents a steeping error of 1.0%.

### Literature Cited

- Anderson, J. A.  
1937 Laboratory malting. I. Equipment. *Can. J. Research* **C15**: 204-216.  
— and Rowland, H.  
1937 Modified equipment and methods for the routine malting test and a study of its precision. *Sci. Agr.* **17**: 742-751.  
Dickson, J. G., Shands, H. L., Dickson, A. D., and Burkhart, B. A.  
1935 Barley and malt studies. I. Developing new varieties of barley for malting and their properties. *Cereal Chem.* **12**: 596-609.  
Meredith, W. O. S., and Anderson, J. A.  
1938 Varietal differences in barleys and malts. IV. Commonly measured properties and their correlations with nitrogen and 1000-kernel weight. *Can. J. Research* **C16**: 497-509.  
Sallans, H. R., and Anderson, J. A.  
1939 Observations on the study of varietal differences in the malting quality of barley, IV. *Can. J. Research* **C17**: 57-71.

# **CONTROL OF DOUGH TEMPERATURE DURING FERMENTATION<sup>1</sup>**

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(Read at the Annual Meeting, May 1939)

Last year in reporting on a co-operative test of a punching and moulding machine (Malloch, 1939), it was suggested that factors other than the manipulation of the dough contribute materially to the variability of test baking. Since temperature has a marked effect on all the processes involved in dough fermentation and on the colloidal behaviour of dough, it seemed logical to examine the adequacy of the control of dough temperature at the various stages of test baking, and the fermentation stage was chosen as a starting point.

## **Dough Temperatures in a Fermentation Cabinet**

Experiments were conducted to check the consistency of dough temperatures in the fermentation cabinet in use in this laboratory. The cabinet is an air thermostat similar to that described by Larmour, Machon, and Brockington (1931) operated at the normal temperature of 30° C., in which the doughs are fermented in covered earthenware crocks. The doughs under study were placed in these crocks and four thermocouples were inserted. Two were pushed well into the centre of the dough from the bottom and the top respectively and the others were located in the outer layer at either side. A fifth thermocouple was placed in the cabinet close to the crock. Measurements were made periodically without punching the dough or opening the door of the cabinet.

In the first experiment the dough was mixed at approximately 28° C., and on standing in the cabinet the temperature gradually rose until it reached cabinet temperature after 70 minutes of fermentation. The temperature continued to rise until at 90 minutes it was nearly half a degree above cabinet temperature. After the first 30 minutes, variation between the different parts of the dough was small. The results are shown graphically in Figure 1.

In the second experiment (Fig. 2) the dough was mixed above cabinet temperature. A reading taken by thermometer immediately after mixing showed 32° C. The temperature of the dough rose slightly at the beginning of fermentation and then fell steadily to the

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<sup>1</sup> Published as Paper No. 169 of the Associate Committee on Grain Research of the National Research Council of Canada and the Dominion Department of Agriculture. Contribution to the Report of the 1938-1939 Committee on the Standardization of Laboratory Baking. N. R. C. No. 870.

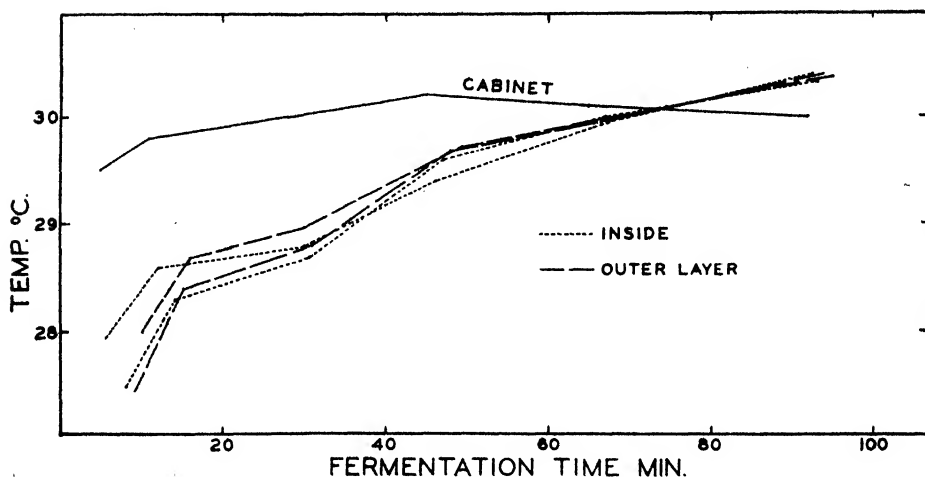


Fig. 1. Temperature of a dough mixed below cabinet temperature.

end of the experiment but had not reached cabinet temperature at 150 minutes. As the temperature fell the spread between the outside and the inside of the dough decreased but at 150 minutes the outer couples were  $0.5^{\circ}\text{C}$ . above the cabinet temperature while the inside of the dough was  $1^{\circ}$  above.

The air thermostat failed to bring the doughs to, and to maintain them at, the required temperature. It therefore cannot be relied upon to correct for the differences in temperature after mixing which are found between flours of different characteristics. Three factors are responsible for the unsatisfactory results. The heat transfer between a solid and the surrounding air is notoriously bad. The fer-

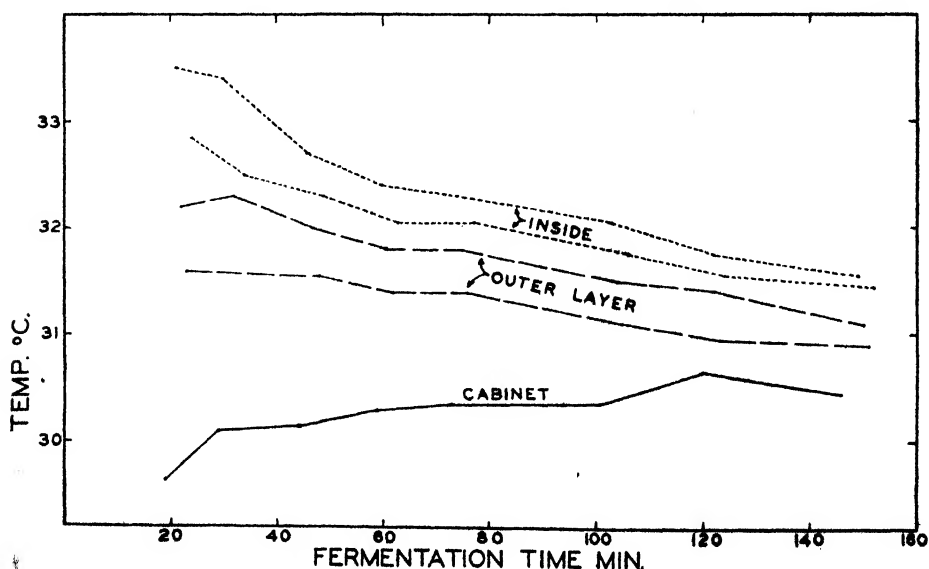


Fig. 2. Temperature of a dough mixed above cabinet temperature.

mentation reaction is exothermic and efficient transfer is necessary for the removal of the heat generated. Dough has a fairly high insulating value because of its porous structure and the heat transfer from one part of the dough to another is relatively slow. The temperature of the cabinet was reasonably uniform throughout each experiment and, while some improvement in heat transfer might be accomplished by forced-air circulation and by the use of metal containers, it is doubtful if any air thermostat is satisfactory for the control of dough temperatures in test baking or in any other test.

### Comparison of Water and Air Thermostats

To determine whether the variability in test baking results could be reduced by use of a water thermostat to replace the cabinet for storage of fermenting doughs, temporary equipment was constructed. It consisted of ten metal containers suitably supported, immersed in a water bath, and provided with loose covers. Three flours were used in the experiment. Each of these flours was baked on ten different days. On each day ten doughs were fermented in the new equipment and ten in the old cabinet. Three extra loaves were baked at the beginning and end of the series and discarded. Thus the results of 300 bakings using each method of temperature control were available for calculation of the variability.

In previous studies of experimental baking in this laboratory (Malloch and Hopkins, 1935) it was not possible to combine the results obtained on different days because of the instability of the variance. The daily variability in the present experiment was calculated and found to be reasonably constant. Only three of the sixty groups gave standard deviations which were significantly different from the appropriate average of the daily deviations. In two of these cases the increased variability can be attributed to the abnormal volume of a single loaf; in the third, two loaves were concerned. Since the variance is reasonably stable it is possible to consider all the results for each flour together. The variability of each flour by the two methods of temperature control is given in Table I.

TABLE I  
EFFECT OF FERMENTATION IN WATER BATH ON VARIABILITY IN LOAF VOLUME

Flour	Standard deviation	
	Cabinet	Water bath
	cc.	cc.
A	15.9	13.6
B	15.9	13.7
C	14.5	12.8

There was a reduction in variability by use of the water bath with all three flours. The values given include variations from all sources, between days, within days, and random error. The data were subjected to an analysis of variance to ascertain the distribution of the variability between these sources. The results of this are summarized in Table II.

TABLE II  
ANALYSIS OF VARIANCE

Variance due to	D.F.	Mean square flour A		Mean square flour B		Mean square flour C	
		Cabinet	Bath	Cabinet	Bath	Cabinet	Bath
Days	9	.092 <sup>1</sup>	.066 <sup>2</sup>	.097 <sup>2</sup>	.076 <sup>2</sup>	.136 <sup>2</sup>	.117 <sup>2</sup>
Replicates	9	.015	.037 <sup>2</sup>	.034 <sup>1</sup>	.016	.023 <sup>2</sup>	.022 <sup>2</sup>
Random error	81	.019	.011	.016	.013	.008	.005
Standard error ( <i>cc.</i> ) (calculated from random error)	—	13.9	10.6	12.8	11.4	8.9	6.8

<sup>1</sup> Exceeds 5% point.

<sup>2</sup> Exceeds 1% point.

The differences between days are significantly greater than the random error in all series. However, the water bath gives more constant results. In four series there was a systematic error within days (referred to as "replicates" in the table). This arises from the characteristically low or high values obtained for loaves of the same number in the series throughout the different days baking. With flour A this systematic error was higher when the bath was used but the reverse was true of the other two flours. The loaves fermented in the water bath gave a lower random error with all flours.

The original data were examined to find the source of the systematic variations within and between days. The pertinent results are summarized in Table III.

Throughout the entire experiment the values obtained on the first day on which each flour was baked were characteristically low. This is almost entirely responsible for the significant variance "between days." Since none of the flours were baked on ten successive days and the flours were baked at different times it is difficult to see that this behaviour can be explained on the basis of consistent differences in the equipment or baking technique on those particular days. The explanation that the equipment had not been in continuous use is certainly not valid. The only possibility which remains is that the sampling of the bulk of flour was faulty, and this may have been the case. In

TABLE III  
SOURCE OF SYSTEMATIC VARIATIONS

Flour	Treatment	Loaf volume		
		General mean	Mean, Day 1	Mean, Loaf 1
		cc.	cc.	cc.
A	Cabinet	646	627	646
	Bath	653	636	647
B	Cabinet	752	738	740
	Bath	750	734	749
C	Cabinet	651	622	646
	Bath	662	636	652

the four series where there were significant systematic differences between replicates, the first loaf of the series was characteristically lower in volume than the general mean. It is possible that the three extra loaves which were included with each series were not sufficient to allow the equipment and particularly the oven conditions to become stable. The inclusion of four or more extra loaves would probably have reduced the variability in the experimental series.

### Discussion

It is evident from these results that the conventional fermentation cabinet is not satisfactory equipment for controlling dough temperatures and that the use of a water bath for this purpose reduces the variability. A further reduction can probably be made when permanent equipment is constructed as the covers of the containers used in this experiment did not fit uniformly well and there were differences in the degree of skinning of the doughs, which probably affected the loaf volume.

The systematic errors found in this experiment are relatively small but nevertheless they will be investigated with a view to their removal. The random error has been reduced to a point only a little above a satisfactory level. Attempts will be made to reduce this error still further. The success of improved temperature control at one stage of baking in reducing the variability makes an examination of the possibilities of improving other stages highly desirable. This work is now in progress in this laboratory. A water-jacketed mixer has been constructed, a suitable proof box has been designed and is under construction, changes have been made in the wiring of the oven, and alterations are being made in the air-conditioning equipment to give improved control of temperature.



### Literature Cited

- Larmour, R. K., Machon, F. D., and Brockington, S. F.  
1931 A fermentation and proofing cabinet giving low temperature variability. *Cereal Chem.* 8: 233-242.
- Malloch, J. G.  
1939 Co-operative test of a punching and moulding machine. *Cereal Chem.* 16: 29-32.
- and Hopkins, J. W.  
1935 Variability in experimental baking using hand and machine manipulation. *Cereal Chem.* 12: 57-61.

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## PREDICTION OF BAKING VALUE FROM MEASUREMENTS OF PLASTICITY AND EXTENSIBILITY OF DOUGH. I. INFLUENCE OF MIXING AND MOLDING TREATMENTS UPON PHYSICAL DOUGH PROPERTIES OF TYPICAL AMERICAN WHEAT VARIETIES

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The physical properties of the gluten complex of a dough are in a continuously changing state, the rate of change being contingent upon the treatment accorded to the dough, its age, the proportion of water used for its preparation, and other variables. In such a complex and heterogeneous dynamic system, a single measurement of a physical property or properties is of limited value. A record of the continuously changing state of the dough as a function of the customary dough treatments including mixing, resting, molding, etc., should prove much more valuable.

The farinograph is adapted to the measurement of dough plasticity as a function of continuous mixing. This instrument has also proved successful in estimating the capacity of a strong flour to yield doughs of acceptable properties when blended with weak flour. From the characteristics of farinograms other dough properties, such as the rate of hydration, sensitivity to mixing, and buckiness, can be estimated. The farinograms are of limited value in certain other particulars, however. Thus they fail to reveal the direction and magnitude of the effect of chemical treatments, or the recovery or tendency of the dough to regain certain of its original properties after excessive mixing. It has been shown recently that these treatments are mainly registered in doughs at rest. In this condition doughs exhibit distinctly different properties from those shown when they are in an "excited" condition, as effected for instance by mixing, molding, or other dough manipulations. We probably deal here with a process analogous to "work hardening" known in other fields of physics.

To measure the strain and stress relationship of doughs at rest it was necessary to return to measurements of extensibility. Such measurements have been made at times in the past when the dough was still in an "excited" state, that is, when it was freshly mixed or shaped. An instrument recently devised by Brabender and known as the extensograph (Fig. 1) provides opportunity for determining extensibility after permitting the dough to rest for suitable intervals of time. In this paper the effects that physical treatments of the dough, such as mixing and molding, have upon the dough properties that are involved in extensibility, will be shown.

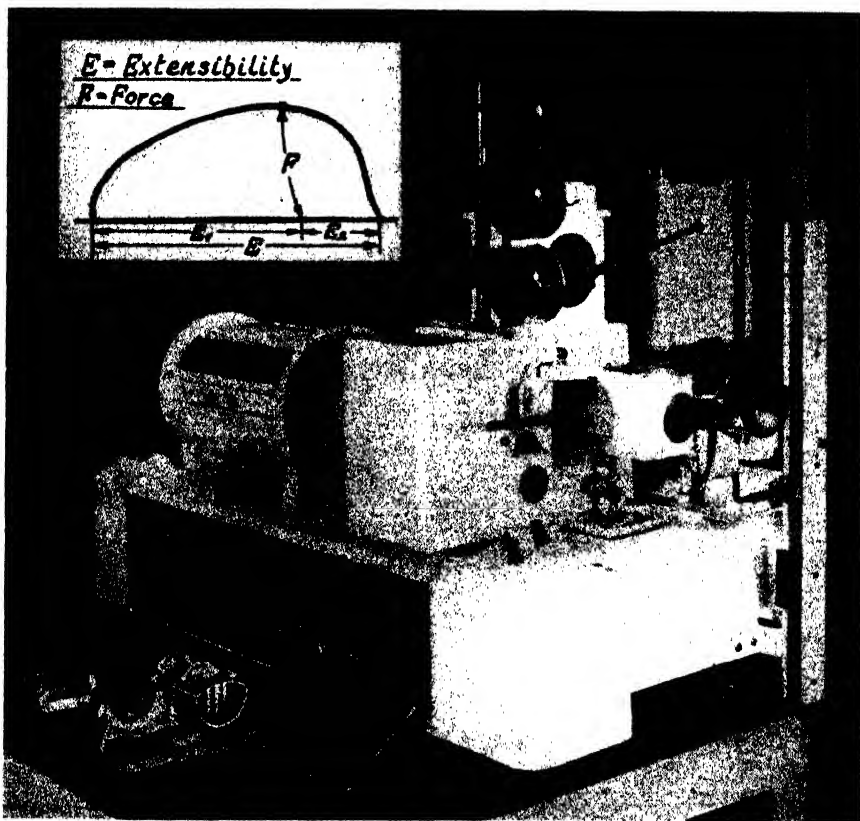


Fig. 1. Photograph of extensograph, with insert of schematic extensogram.

In these studies the doughs were prepared in the following standard manner: 300 g. of flour plus 6 g. of salt were mixed in the farinograph to a consistency of 500 units. After mixing for exactly 5 minutes the machine was stopped, the dough removed, and two portions of 150 g. each were rounded up by means of the device that is a part of the extensograph ensemble. These smoothly rounded masses were passed once through the molder that is provided with the instrument, and were then clamped firmly in the dough holder. They were placed in

the thermostat or fermentation cabinet for 60 minutes, unless otherwise indicated, and then subjected to extensibility measurements. This treatment was repeated at intervals as described in the later portions of this paper. Extensogram constants computed and recorded in certain of these studies are referred to by symbols as follows (Fig. 1):

$F$  = force applied to extend the dough at constant speed of extension, measured along the vertical axis of a typical extensogram, one unit being equal to 1.6 g.<sup>1</sup>

$E$  = extensibility, 1 cm. (10 units) on the horizontal axis of the diagram corresponds to an equivalent dough extension of the original dough length. For instance at 10 cm. the dough is extended about 10 times its original length.

$E_1$  = length of extension at optimum point of resistance to extension or force.

*Velocity of graph paper* = 6.5 mm./sec.

*Velocity of dough hook* = 13.6 mm./sec.

*Area* = area under the extensogram recorded in units of 0.1 cm<sup>2</sup>.

### Changes in Stress/Strain Relationship of Doughs as a Function of Rest Time

It is common baker's knowledge that doughs from various flours respond quite differently to fermentation and to the customary dough treatments during fermentation, the differences being evident not only in gas production but in physical dough characters as well. Cereal chemists have studied such relations quite extensively by means of the baking test, but exact physical measurements had so far not been reported, mainly because of lack of suitable apparatus. The extensograph enables certain work to be done in this direction. Studies were conducted on (a) fermenting doughs, using various amounts of yeast; (b) doughs containing no yeast and only 2% salt (regular procedure); (c) doughs containing 2% salt and lactic acid sufficient to obtain a pH of 5.4.

Since the direction of changes during resting or fermentation were the same in all three procedures, we shall report here for the sake of brevity only the experiments of the series b.

Changes in dough structure of a strong and a weak flour were studied in three stages after mixing, namely immediately, and after two and four hours of rest, respectively. In each instance the molded masses of dough were allowed to stand for varying intervals up to 120

<sup>1</sup> The standard procedure used at Duisburg at the present time deviates in the following points:  
<sup>a</sup> (a) one unit of resistance to extension corresponds to a force of 1.25 g.; (b) time of rest is 45 minutes;  
(c) doughs are mixed one minute, rested 5 minutes, and mixed for another 3 minutes.

minutes and the extensograms were made from time to time during this period. The area and the ratio  $F/E$  were then computed, with the results expressed graphically in Figure 2 for the strong flour, and in Figure 3 for the weak flour.

In Figure 2, involving a dough made from strong American spring-wheat flour, it is apparent that its extensibility increased and the resistance to extension decreased when the dough was permitted to stand for a long time after it had been excited by molding. As the dough grew older, *i.e.*, when the molding was done two or four hours

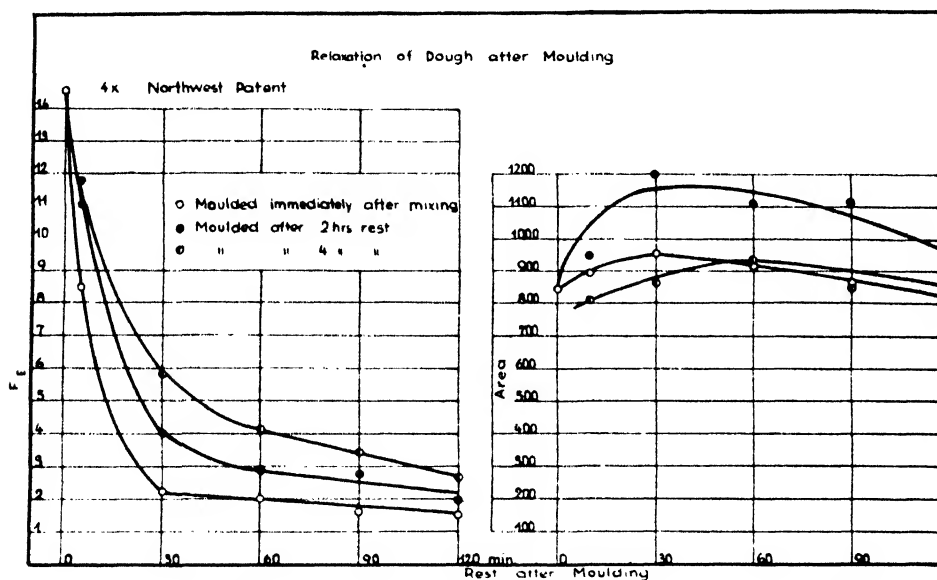


Fig. 2. Change of  $F/E$  relationships and of areas under extensograms on resting of dough made from strong flour.

after mixing, the relaxation from the excited state as induced by molding proceeded at a slower rate. Thus when the comparisons were made 30 minutes after molding, the  $F/E$  ratio of the freshly mixed dough had receded to 2.2, while the  $F/E$  ratio of the four-hour dough was 5.8.

The weaker European blend involved in the studies, recorded graphically in Figure 3, yielded a dough which behaved entirely differently. The  $F/E$  ratios of the freshly mixed and the two-hour and four-hour doughs did not materially differ when compared 30 or 60 minutes after molding. In fact such differences as were encountered appear to be in the reverse order from the strong-flour doughs, since there was evidence of an increased rigidity in the structure on aging of the latter. It will be demonstrated in a succeeding article that the change in relaxation on aging is not only dependent upon the kind of flour and variety of wheat but also upon the customary chemical treatments or the age of the flour.

The term "relaxation" as used above is not quite analogous to the Maxwell's "relaxation time" discussed by Schofield and Scott Blair. Halton and Scott Blair (1936a, 1936b) associated this term with "spring" and arrived at it by separate determinations of dough viscosity ( $\eta$ ) and shear modulus ( $n$ ).

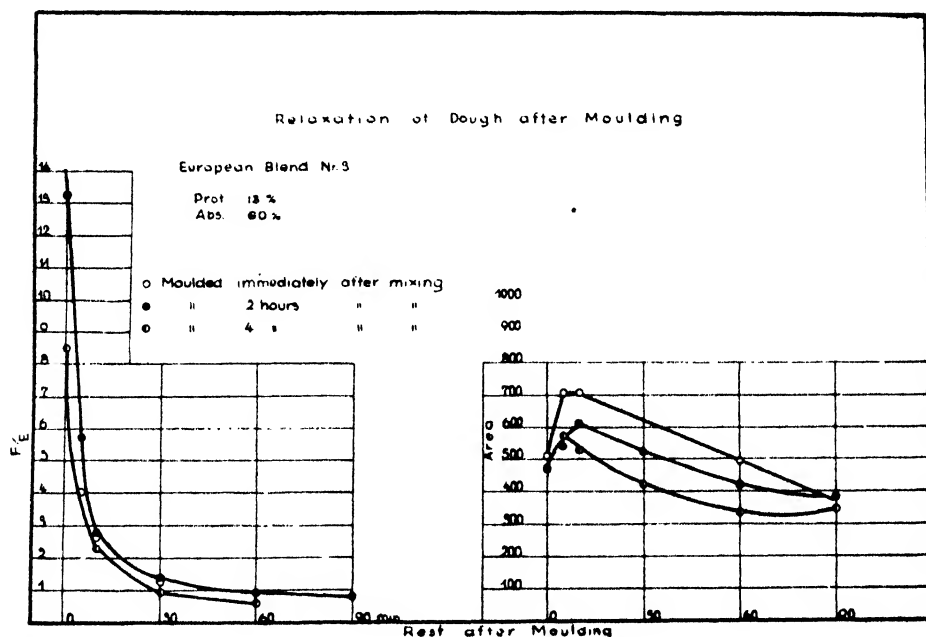


Fig. 3. Change of  $F/E$  relationships and of areas under extensograms on resting of dough made from weak flour.

### Change in Dough Extensibility with Extended Mixing

Bohn and Bailey (1936a, 1936b) reported that stress readings taken at different stages during dough mixing were proportional to the corresponding consistency values of the farinograph. The hysteresis effects of different degrees of dough mixing were not included in their studies. We devoted our attention especially to the effect of mixing and to aged doughs.

Practical baking experience has generally resulted in the conclusion that fermentation time may be reduced by extending the mixing time.<sup>2</sup> The study of fermentation tolerance is thus dependent upon a complete knowledge of the kneading or mixing treatment. Furthermore, a discussion of the "optimum" in the instance of either mixing time or fermentation time would involve a specification of the other variable. Flours can be "mixing sensitive" because of weak gluten structure, where the gluten is actually damaged by overmixing or tight gluten character, in which overmixing accentuates buckiness. In the latter

<sup>2</sup> Exceptions have been noted in the instance of certain Canadian flours.

case mixing sensitivity is usually proportional to sensitivity towards oxidizing agents.

Under conditions such as prevail in bakeries in the United States the first kind of mixing sensitivity is encountered mainly in some of the weaker southwestern wheat varieties; for instance Blackhull. An extensive study of the mixing tolerance of southwestern flours revealed that if flours of about equal protein content showed decided differences in their respective farinograms, such differences were reflected again in the mixing sensitivity of these flours. In these experiments mixing was done by means of a Hobart vertical high-speed mixer, and the other baking operations were carried out on a semi-commercial basis. The fermentation time was varied from three to five hours, and the doughs were proofed to standard height. Mixing intensity was limited to three degrees, namely:

<i>For normal mixing</i>	1 minute, low speed
	1 minute, medium speed
	2 minutes, high speed
<i>For overmixing</i>	4 additional minutes, high speed
<i>For severe overmixing</i>	8 additional minutes, high speed

The flours were classified into the following three groups according to their protein content:

- Group A* = 13.0–15.0% protein
- Group B* = 11.5–13.0% protein
- Group C* = 10.5–11.5% protein.

Each group was divided into three sub-groups according to general strength rating on basis of the farinogram. Besides the general appearance of the curve, the "developing time" served mainly as criterion of classification, thus:

- Sub group I* had 9–12 minutes "developing time"
- Sub group II* had 6–9 minutes "developing time"
- Sub group III* had 6 minutes "developing time."

Subgroup *I* stood severe overmixing very well, while subgroup *II* was slightly and subgroup *III* severely damaged, a fact which showed up especially at longer fermentation times. In the high-protein group overmixing was not quite as injurious as in the two lower-protein groups.

Looking at the problem from the standpoint of the commercial baker it seems that the hazard of damage to the gluten structure on account of overmixing might be limited to flours of subgroup *III*. The commercial baker mixes to the dry point, and sometimes continues

a little beyond if he desires to raise the temperature of the dough. Such drastic mixing as was accorded in our experimental studies does not occur in such commercial practices.

Mixing sensitivity because of tight gluten structure is probably encountered more frequently. We have therefore given this problem special attention. Two flours of about equal protein content, 12.7% and 12.5%, marked *GM3* and *GM5* respectively, afforded an interesting comparison. From their farinograms, recorded in Figure 4, it might

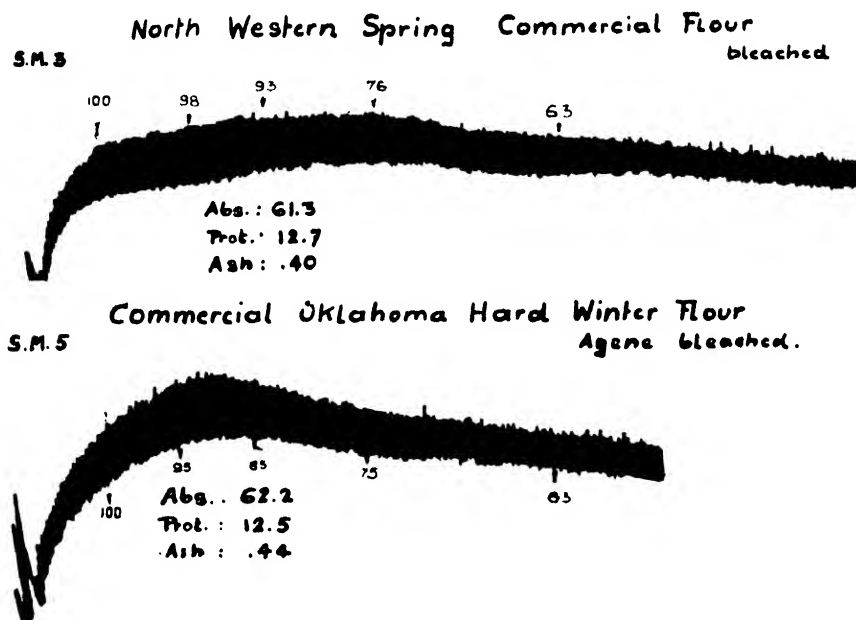


Fig. 4. Farinograms of *GM3* and *GM5*.

be assumed that the northwestern spring-wheat flour *GM3* would evidence greater mixing resistance than the Oklahoma or southwestern flour *GM5*. To test this assumption, doughs made from both flours were subjected to varying mixing treatments, being removed from the farinograph mixer after 3, 5, 7, 10, and 15 minutes of mixing, respectively. These doughs were then subjected to tests in the extensograph after having been permitted to rest for varying intervals up to 7 hours. The results of these tests, together with a graphic record of the volume in cc. of test loaves fermented for varying periods as shown, are recorded in Figures 5 and 6. In order to eliminate the gas-production factor as much as possible 5% of sugar and 0.25% of diastatic wheat malt flour were added to the dough. Otherwise the A.A.C.C. standard baking test method was followed with the exception that 1 $\frac{3}{4}$ % of yeast was used. This formula should provide for sufficient gas production up to 4 hours of fermentation.

From Figure 5 it is apparent that when the southwestern wheat flour dough, accorded extended mixing (15 minutes), was promptly tested for extensibility, the area of the extensogram was reduced substantially below that of the dough mixed for 3 or 5 minutes; for example, the curve area for the 15-minute mixing period is only 230 units as compared with 900 units for the three-minute mixing time. When the dough mixed for 15 minutes was permitted to rest for several hours after mixing, however, the area of the extensogram increased markedly,

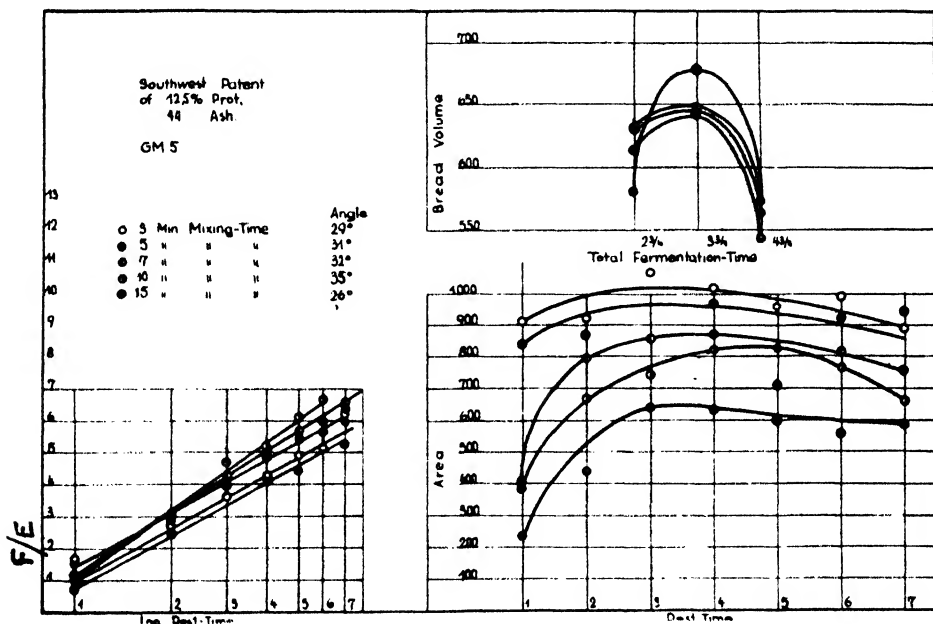


Fig. 5. Changes in  $F/E$  relationships of extensograms, plotted against the logarithm of resting time of doughs made from GM5; area under extensograms of doughs on resting; loaf volume in cc. of bread baked from doughs fermented for varying time intervals.

reaching 630 units after 3 hours. A somewhat similar behavior was observed in the instance of the dough mixed 7 and 10 minutes respectively. At the same time the general shape of the curve was altered, as evidenced by the  $F/E$  ratios recorded graphically at the left of the figure.

These changes in area are in sharp contrast with the behavior of analogous doughs prepared from the northwestern wheat flour GM3. In this instance the dough mixed for a long period (15 minutes) gave an extensogram of larger area (520 units) than the corresponding southwestern wheat flour dough, but there was little increase in area on standing, and at the end of three hours the area was actually less in the northwestern than in the southwestern flour dough. These differences in the extensograms are well supported by the practical baking test. Thus in Figure 7 where photographs of the test loaves are shown, it is



evident that the southwestern flour doughs (marked *S* on the loaves) recovered from the heavy mixing treatments on fermentation and gave larger loaves of good texture than the northwestern wheat flour doughs (marked *N* on the loaves).

Thus in our experiments the northwestern flour dough is more mixing-resistant only when the doughs are tested one hour, or, at a maximum, two hours after the mixing process. Later the gluten tightening has proceeded to such an extent that the dough becomes too short for a large extensograph area or for normal baking behavior.

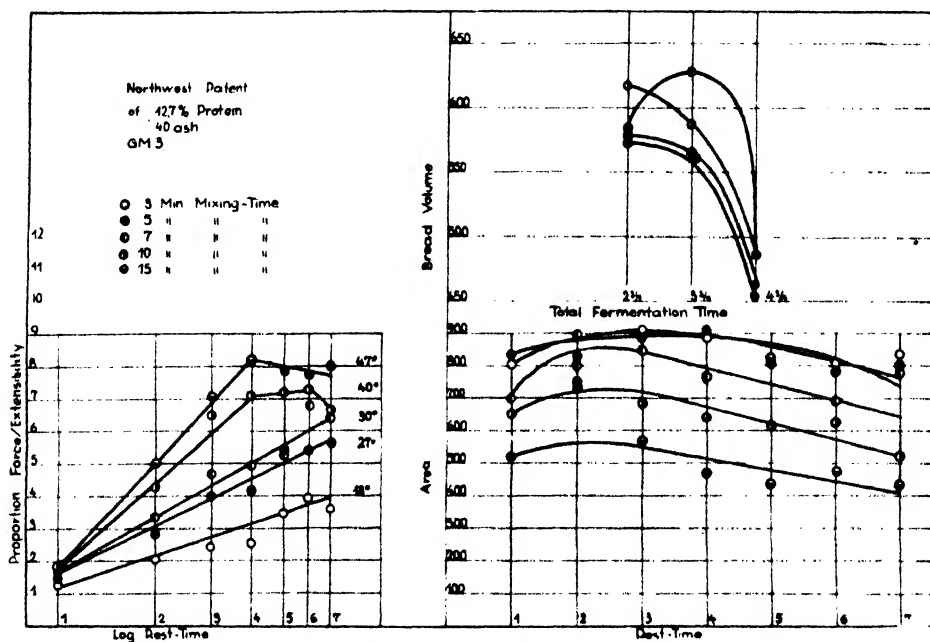


Fig. 6. Changes in  $F/E$  relationships of extensograms, plotted against the logarithm of resting time of doughs made from *GM3*; area under extensograms of doughs on resting; loaf volume in cc. of bread baked from doughs fermented for varying time intervals.

The tightening of the gluten structure is suggested by the progressive change in the  $F/E$  ratio, which is plotted against the logarithm of time in hours in Figure 6. Note that these graphs approach a straight line for the first four hours, and that they diverge from one another in the instance of the several doughs accorded different mixing treatment. Among the corresponding southwestern flour doughs such divergence is much less apparent. Moreover, the angles which these graphs form with the horizontal or axis of abscissae are greater in the northwestern flour doughs at 10 or 15 minutes of mixing, which affords added confirmation of the greater tightening action resulting from extended mixing.

Accordingly it is concluded that the slope of the farinograph curve after the point of maximum consistency is not always a measure of

mixing tolerance. This is only the case when one deals with weaker flours of similar character, as for instance in the comparison of the different southwestern wheat varieties.

The differences obtained in our experiments are probably somewhat exaggerated through the particular action of the farinograph mixer.

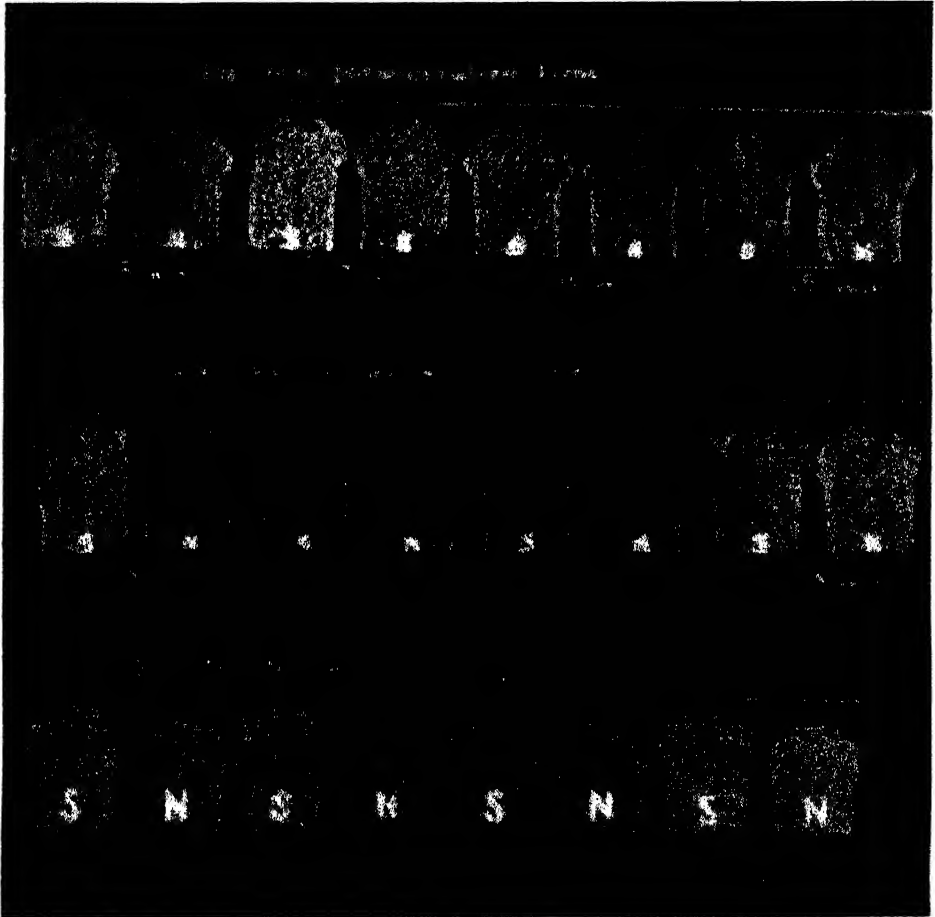


Fig. 7. Photographs of loaves baked from southwestern wheat flour *GM5* (marked *S*) and north western wheat flour *GM3* (marked *N*).

It is possible that the customary high-speed mixer will not give differences of the same magnitude. Furthermore the flours were already several months old at the time of testing and therefore already somewhat short in character. Since we are discussing fundamentals rather than evaluating flours, the magnitude of the differences does not play a big role, however.

While the increased tightening effect on the gluten with extended mixing sufficiently explains why a stronger flour can actually be less

"mixing resistant," there is still the question as to why the high-speed mixer proved to be especially successful on the tougher and stronger northwestern flours. The question is: Does high-speed mixing actually provide more extensive mixing treatment, or does it develop slacker doughs better than is possible with slow-speed mixing? From the observation that stiff doughs are developed better than slack doughs in the farinograph mixer (other slow speed mixers produce similar effects), even if the total work input is the same in both cases, it is concluded that the alternating intensity of compression is one of the major factors

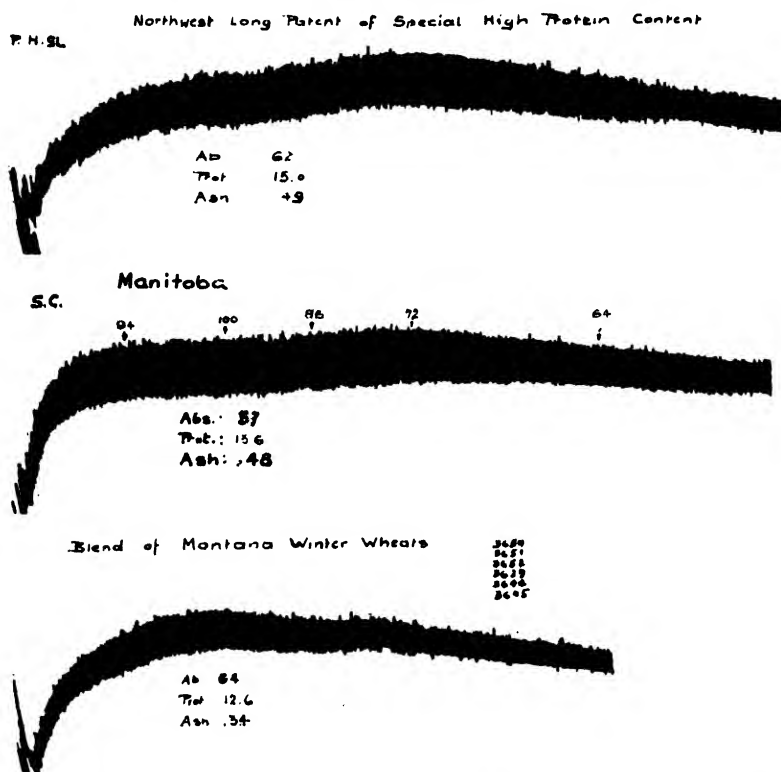


Fig. 8. Farinograms of high protein northwestern spring-wheat patent, Manitoba S.C. and Montana winter wheat flours.

in dough development. Slow-speed mixers do not seem to develop enough compression on slack doughs. Actually about 2% more water is incorporated by the Hobart high-speed mixer (two-gallon bowl) than by the farinograph mixer, on the basis of the same farinograph consistency. Furthermore a dough developed in the high-speed mixer gives a farinogram with a wider band than one developed from the beginning in the farinograph mixer. Since the northwestern flours tighten up considerably during fermentation, the development of slack dough becomes of major importance. It is therefore believed that the high-speed mixer was successful, mainly because it develops slacker dough more

efficiently, thus giving a smoother and more elastic dough, and not because of added or more intensive mixing treatment.

Some other observations seem to indicate that the farinograph and some other types of slow-speed mixers incorporate more air ( $O_2$ ) into the dough. This is analogous to added oxidation treatment, which is usually detrimental to the already tough spring-wheat flours.

Flours *GM3* and *GM5* afforded means of comparing such types of flours, since they give farinograph curves of nearly equal "developing time" (mixing time to the point of either decrease in curve width or

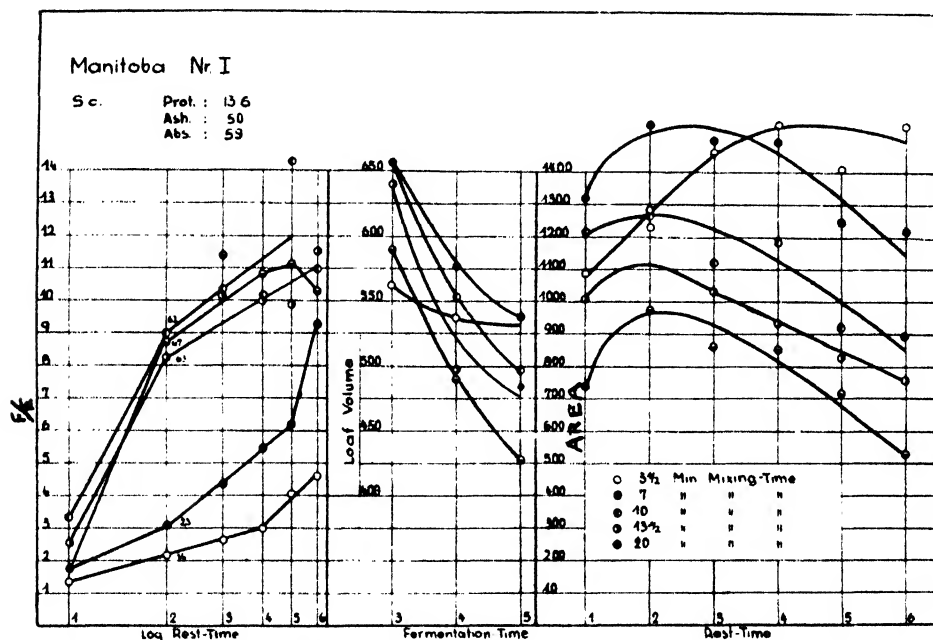


Fig. 9. Changes in  $F/E$  relationships of extensograms, plotted against the logarithm of resting time of doughs made from Manitoba SC: area under extensograms of doughs on resting; loaf volume in cc. of bread baked from doughs fermented for varying time intervals.

decrease in consistency—in the case of *GM3*  $6\frac{1}{2}$  minutes and in the case of *GM5* 6 minutes) but they differ in the slope of consistency decrease after this point. The northwestern long patent and the Manitoba flour, the farinograms of which are shown in Figure 8, afford a comparison of flour types of equal "developing time" and equal consistency decrease after this point, being different however in the slope of the initial part of the farinograph curve. Extensograms of doughs mixed for increasing intervals of time, and allowed to rest for periods up to five or six hours, disclosed a greater sensitivity to overmixing of the Manitoba flour SC. Thus after five hours of rest the extensogram area (Fig. 9) of the dough mixed 20 minutes was about 850 units less than that of the dough mixed  $3\frac{1}{2}$

minutes. The corresponding decrease for the northwest long patent *PHG1* (Fig. 10) was only about 450 units, or less than half as much. The loaf volumes of test loaves baked from doughs mixed for varying periods of time are in sharp contrast in the instance of these two flours and support the extensograph tests. Likewise the  $F/E$  ratios recorded as a function of the log of time show a decided difference between these two flours. Thus the angle, used here to express the change in dough tightening with time, increased  $40^\circ$ , from  $23^\circ$  to  $63^\circ$ , with the Manitoba flour and only  $13^\circ$ , from  $22^\circ$  to  $35^\circ$ , with the northwestern long patent, when the mixing time was increased from 7 to 10 minutes.

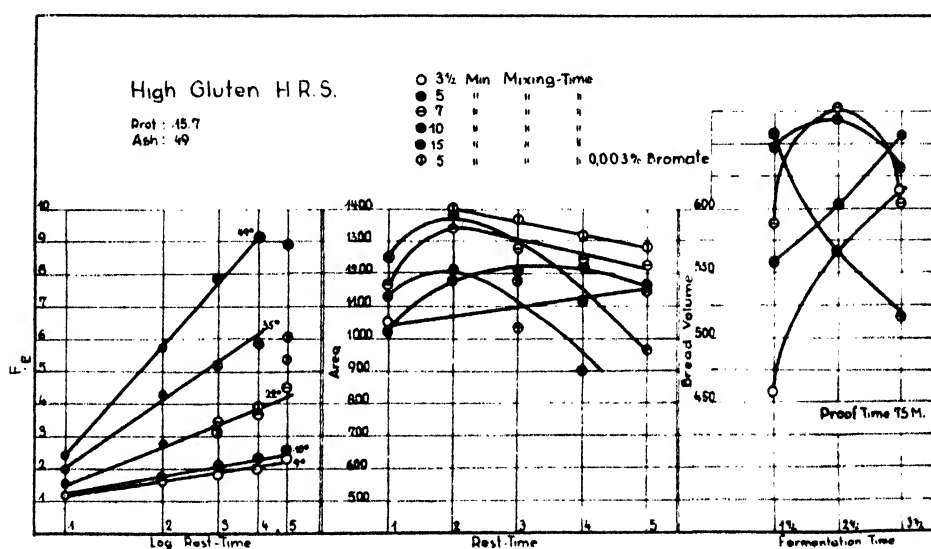


Fig. 10. Changes in  $F/E$  relationships of extensograms, plotted against the logarithm of resting time of doughs made from high-protein northwestern spring wheat patent; area under extensograms of doughs on resting; loaf volume in cc. of bread baked from doughs fermented for varying time intervals.

It has been known of course that fast initial rise of the farinograph curve is indicative of compact gluten character in fairly strong flours, but it usually was not sufficiently realized that doughs of this character are tightened to such a degree by overmixing. Considering the bucky dough problem from this angle it is realized at once why "trouble shooters" always find it best to give such flours little mixing and few "take-ups," rather than longer mixing time, which evidently would render the dough more extensible.

Similar conclusions can be drawn from mixing experiments carried out with flours experimentally milled from two northwestern wheats, Reward and Ceres. Their farinograms (Fig. 11) indicate Reward to be of relatively tight gluten character. The expected higher mixing sensitivity is evident from the extensibility measurements graphically

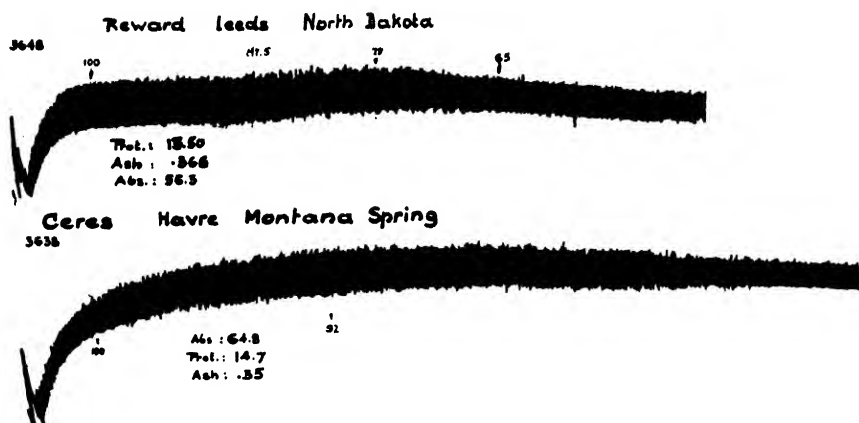
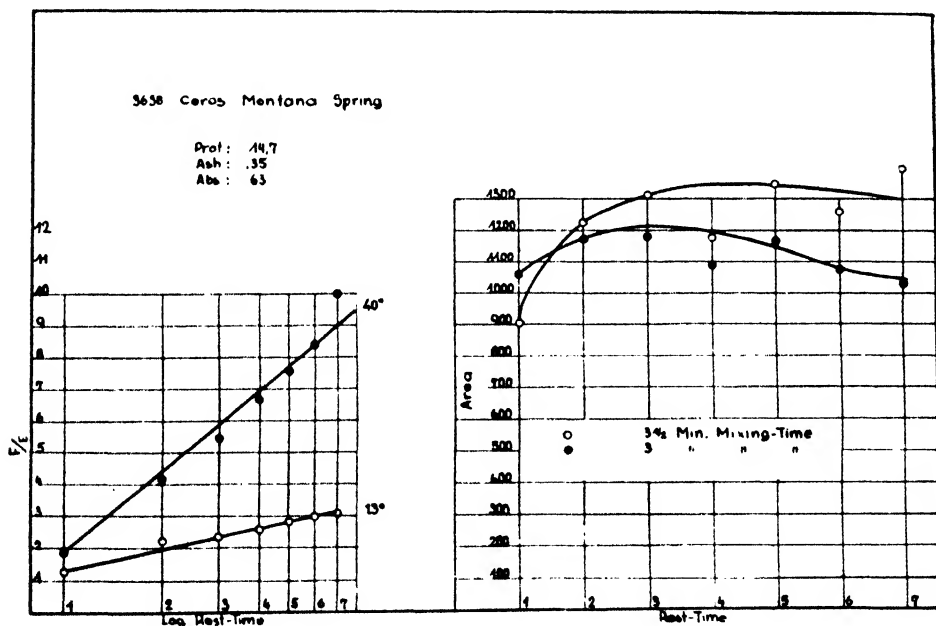


Fig. 11. Farinograms of Reward and Ceres wheat flours.

recorded in Figures 12 and 13. The angle of the  $F/E$  ratio line was for instance  $13^\circ$  for Ceres and  $27^\circ$  for Reward when the doughs were mixed  $3\frac{1}{2}$  minutes in the farinograph mixer.

So far, the cases were quite normal and certain characteristics of the farinograph curve could well be explained in terms of mixing sensitivity and general flour strength. We shall discuss now some types

Fig. 12. Changes in  $F/E$  relationships of extensograms plotted against the logarithm of resting time of doughs made from Ceres wheat and areas under extensograms of doughs on resting.

which at the first moment could be misinterpreted from their farinograph curves.

The Montana winter wheat flour shown in Figure 8 seems to be of normal medium-strong type, if one considers the shape of the curve.

The relatively small curve width is abnormal, however, and together with the relatively high water absorption indicates stickiness of dough. Actually careful observation of the dough itself during the process of mixing disclosed a sticky condition. Still one might expect a medium-large loaf volume on baking it, and an extensogram of equivalent area, especially if its protein content of 12.6% is considered. The extenso-

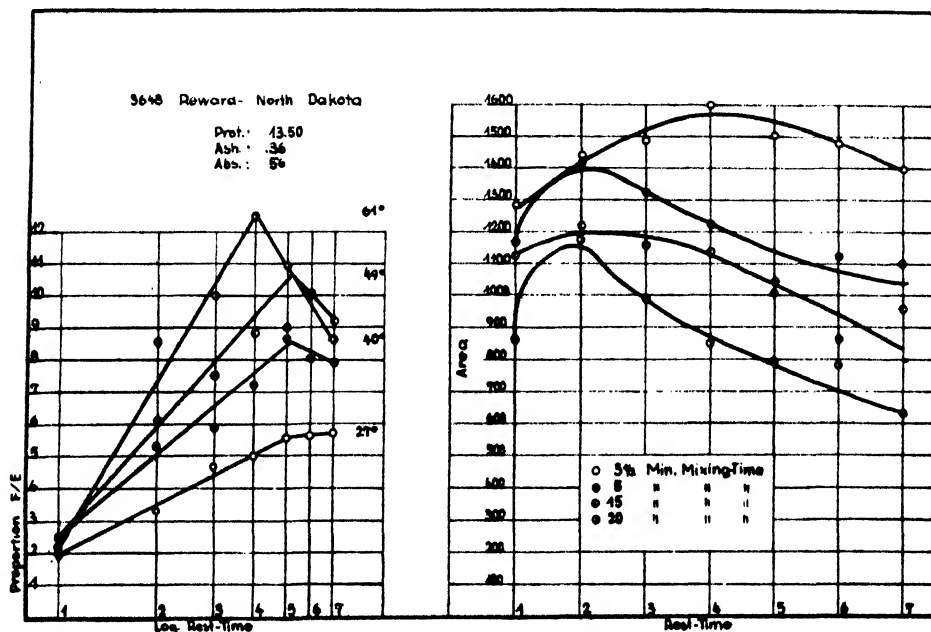


Fig. 13. Changes in  $F/E$  relationships of extensograms plotted against the logarithm of resting time of doughs made from Reward wheat and areas under extensograms of doughs on resting.

gram was medium to small in area, however, as shown in Figure 14, reaching a maximum of only 730 units. The  $F/E$  ratio was very small, and its rate of change with time of rest (plotted as the logarithm) was likewise small, smaller in fact than typical Kansas hard winter wheat flour doughs. The loaf volumes indicate that small changes in the dough structure occurred when the dough was mixed for widely varying intervals of time. Apparently the flour contains an unusually large proportion of substances that hydrate readily, render the dough smeary and sticky, and may even prevent the formation of a normal gluten reticulum. While a narrow farinogram and relatively high absorption give some indications as to the nature of such a flour, they do not afford a basis for an accurate prediction of the baking value. Since baking test and extensograph values coincide, it must be concluded that the effect of such overhydrated substances is more severe in resting doughs than in doughs during kneading.

At times wheat varieties have been tested (especially some Canadian wheats) which gave very strong farinograms, but baked well only after

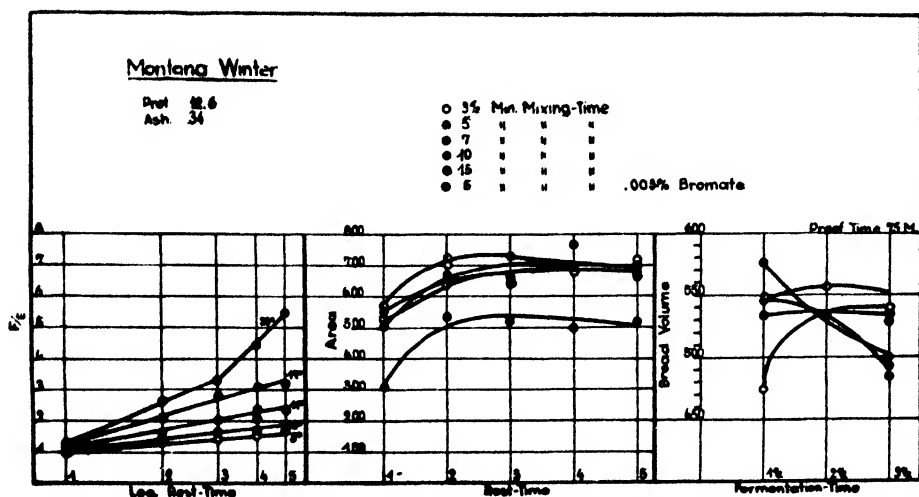


Fig. 14. Changes in  $F/E$  relationships of extensograms plotted against the logarithm of resting time of doughs made from Montana winter wheat; areas under extensograms of doughs on resting; loaf volume in cc. of bread baked from doughs fermented for varying time intervals.

being given a certain oxidation treatment. Resting doughs show a large extensibility and little resistance to extension. This is another case where it seems that some substances, very likely of fat-like nature, may orient themselves between gluten interfaces of doughs at rest. In the oxidized state they seem to lose their potency. The similarity of this type of dough and that prepared from Montana winter lies in the fact that both give stronger farinograms than are anticipated by the baking behavior of the untreated flour; the difference is that the Montana winter type is not improved considerably by oxidizing agents, while the other reacts very strongly. When deductions are made from the farinogram, it therefore must be kept in mind that secondary effects not registered in "excited" doughs may be of predominant influence in resting doughs and actual baking behavior.

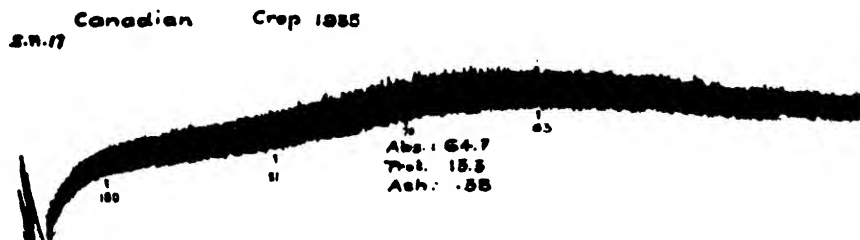


Fig. 15. Farinogram of GM17.

We have demonstrated that a tight gluten structure is registered in the farinogram by a rapidly rising initial curve. If gluten coagulation proceeds still further, one sometimes obtains curves of a general nature as shown by GM17 in Figure 15. At first it appears to indicate a gluten character of pliable and normal elastic behavior, being only different



from that type of curve by the small curve width. The extensogram revealed an extremely tough dough, very sensitive to mixing (Fig. 16). The behavior in the dough mixer might be explained as follows: The coagulation of the gluten proceeded to such an extent that the rate of water absorption of the gluten was retarded considerably beyond the water-absorption rate of normal flours, resulting in a slowly rising curve. On standing, however, the water was bound tighter and the gluten became tough. Slack doughs and little mixing result in a comparatively normal dough with such a flour.

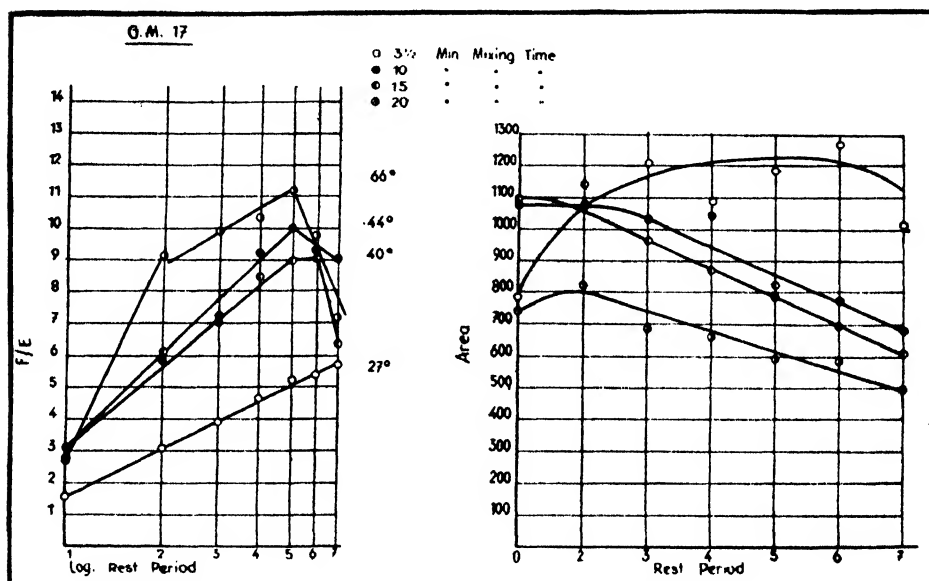


Fig. 16. Changes in  $F/E$  relationships of extensograms plotted against the logarithm of resting time of doughs made from GM17 wheat and areas under extensograms of doughs on resting.

All these observations lead to the following general conclusions as far as deductions of mixing sensitivity from farinograms are concerned: Flours which yield farinograms that are similar except in the initial stages of mixing exhibit differences in sensitivity to overmixing. Flours represented by curve type *A* at the left in Figure 17 will be more sensitive than type *B*. If the fermentation period of the resulting dough is very short ("no-time dough") flour *B* will require a longer mixing treatment than type *A*.

Flours which yield farinograms differing primarily in the *width* of the beginning portion of the curve will vary in sensitivity to overmixing, in the direction of increased sensitivity for the flours having the narrower farinograms (right-hand picture of Fig. 17).

Flours of similar general class (for instance the southwestern hard winter class), differing however in strength as indicated in Figure 18

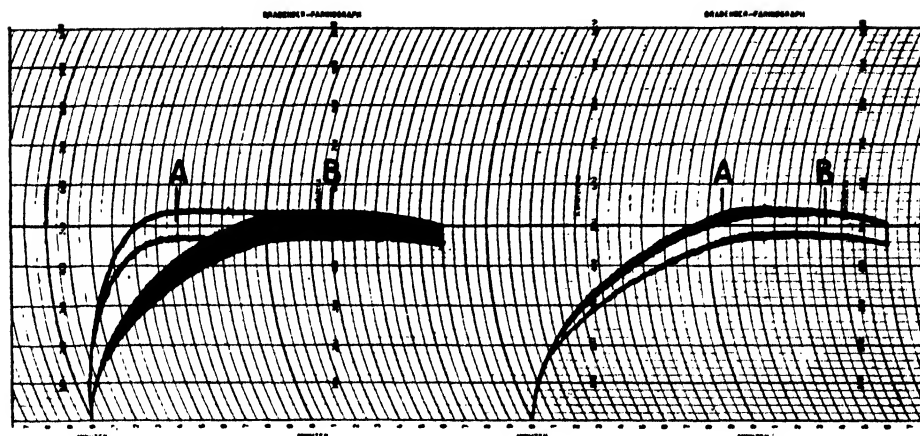


Fig. 17. Schematic farinograms, differing in the initial part of the curve (*A* and *B* at left) and in curve width (*A* and *B* at right).

(*I*), show decreasing mixing sensitivity in the direction of increasing strength, using developing time as a criterion for strength. The difference between curves *A* and *B* of Figure 18 (*I*) actually represents

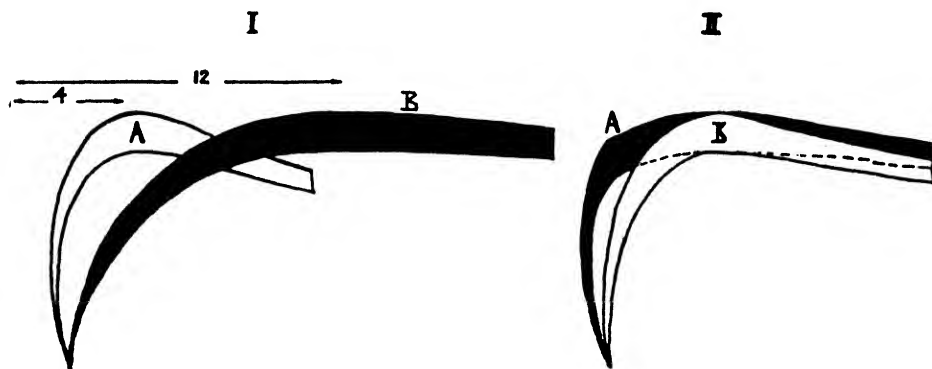


Fig. 18. Character of farinogram as influenced by state of oxidation.

the range of strength observed in the hard winter wheat class of medium-protein level. Under prevailing commercial conditions in the bakery trade the zone where damage by overmixing might be observed is probably restricted to flours of a strength rating of less than six minutes of developing time.

Flours differing in farinogram character as indicated by curves *A* and *B* in Figure 18 (*II*) show differences in mixing sensitivity depending upon the state of oxidation of flour or dough. Usually type *A* will be more sensitive. Type *B* will show a higher capability for dough recovery on standing. Dough recovery is an important factor in appraising American bread flours. A flour which gives a "weaker" farinogram in the sense of a higher "degree of softening" should not

be considered as being more "mixing sensitive," because the rate and magnitude of the subsequent recovery might prove the reverse in many cases. These deductions do not apply in the full sense when "secondary effects" exert a predominant influence.

### Change of Dough Extensibility Effected by Molding

Practical baking experience has indicated that certain types of "bucky" doughs made with strong northwestern wheat flours are more sensitive to molding manipulations than characteristic southwestern hard wheat flours. To determine the occasion for this, as revealed by extensograph measurements, the following studies were undertaken.

Two flours were available for this purpose, a strong Manitoba which yielded a "bucky" dough and a medium-strength Bahia Blanca flour milled from Argentine wheat. In consequence of preliminary observations of these doughs, a schedule of two treatments was laid out which involved (a) molding every hour, and (b) molding only once, one hour before measuring, in the instance of both flours. The results, taken from the extensograms and recorded graphically in Figure 19, show

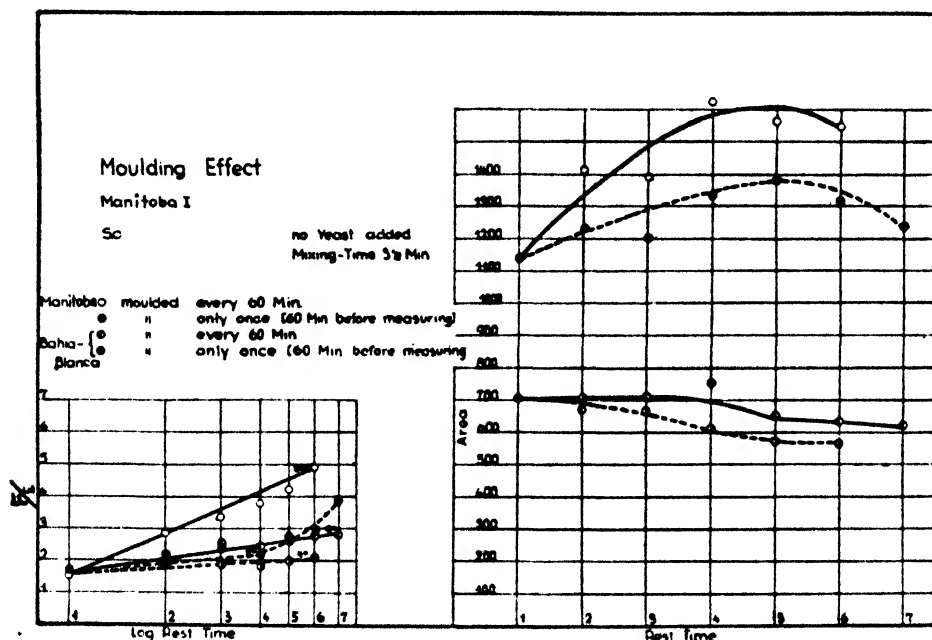


Fig. 19. Changes in  $F/E$  relationships of extensograms plotted against the logarithm of resting time of doughs made from Manitoba and Bahia Blanca wheat and areas under extensograms of doughs on resting.

that the tougher Manitoba flour dough responded much more to the repeated molding than did the more pliable Bahia Blanca dough. Thus the actual increase in the area of the farinogram effected by

molding treatment was twice as large with the Manitoba as with the Bahia Blanca. Likewise, the slope of the  $F/E$  curve plotted against time was greater with the former than with the latter, the values being  $22^\circ$  and  $5^\circ$  respectively.

These extensograms accordingly confirm practical shop experience, since the general practices in baking bucky doughs has been to punch very little and to operate the molder with the sheeting rolls set as far apart as possible in order to secure the best quality of bread. This fact may have some bearing on the methods followed in testing flour with the extensograph. Thus in ordinary bake-shop practice doughs are often punched lightly only once or twice, which is less rigorous than is accorded by the present extensograph "make-up" or rounding and molding apparatus. Differences might become apparent on the extensograms, therefore, which would not appear in the bakery.

To test this assumption further, five flours of rather widely varying strength were tested in two manners, involving (a) molding the dough repeatedly at hourly intervals and testing as heretofore in the extensograph (this being described in Figure 20 as "normal method") and (b)

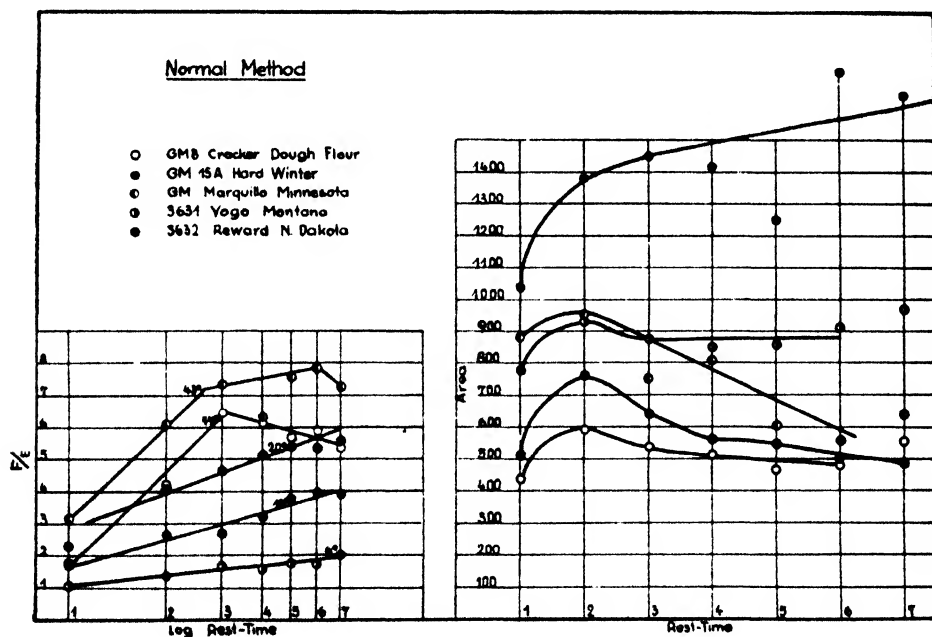


Fig. 20. Effect of repeatedly molding doughs at hourly intervals upon the  $F/E$  ratio and area of extensograms.

dividing the dough from the farinograph into three portions of 150 g. each, molding one piece immediately, the second piece after one hour, the third piece after two hours, and then testing each piece one hour after it was molded. This procedure yielded the extensograph data

recorded graphically in Figure 21. Since with both methods the different flours were rated in the same order, as is apparent from the graphs, we consider the "normal method" the more suitable one. It has the advantage that one is able to make duplicate determinations at every hour and can continue the tests for as many hours as one finds this to be necessary.

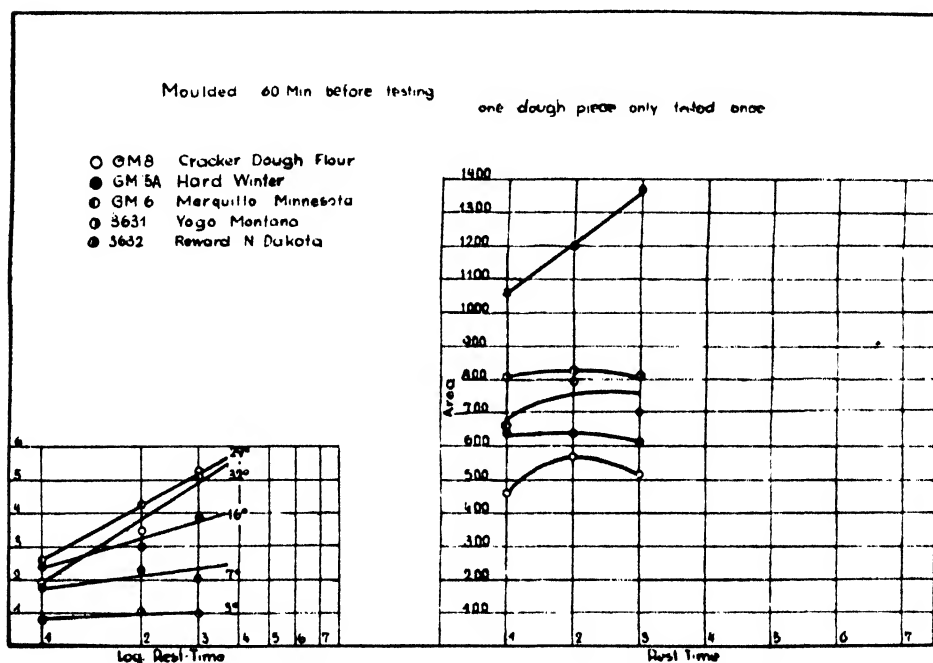


Fig. 21. Effect of molding each piece once after mixing, after one hour of fermentation, and after two hours of fermentation and then testing with the extensograph one hour thereafter, namely one, two, and three hours after mixing respectively.

### Summary

Dough properties change steadily with time and as a function of mixing, fermentation, and other treatments. It appears important, therefore, to study the rate and direction of these changes rather than to measure dough properties at one stage only.

Dough plasticity as a function of mixing treatment is a significant characteristic, but it fails to disclose other important actual or potential properties such as are effected for example by certain chemical treatments. It is not always possible to predict, from such measurements made during the initial mixing, how far a dough may recover its properties after severe overmixing.

Extensibility measurements, such as can be made with the extensograph described in this paper, give useful supplementary information in such instances.

Vigorous treatments such as are accorded by mixing, molding, and punching, effect an "excited condition" in a dough (work hardening), in which condition it exhibits different physical properties from those shown in a state of relaxation. These induced effects do not remain constant, and the dough tends to return to its original state if allowed to stand undisturbed for a time, and the rate and degree of change depend upon the properties of the flour and the treatments to which it, or the dough, has been previously submitted.

On extended overmixing, doughs tend to lose extensibility ( $E$ ) and to increase in resistance to extension ( $F$ ) on standing. This may be analogous to the reversible thixotropic behavior observed in certain simple gels. In general those flours which evidence the greatest sensitivity to overmixing in the dough stage also exhibit the greatest tendency to recover the properties of a normally mixed dough.

Successive extensograph tests conducted on one dough aliquot after varying rest periods gave essentially the same relative results for different flour doughs as tests conducted on separate aliquots for each rest period.

While the ideal procedure in measuring mixing sensitivity with the extensograph is to accord various mixing treatments to a series of doughs prepared from the same flour and then observe the progressive changes in properties with time, the general class of flour into which most American bread flours fit can be recognized, usually, from the farinograms. Accordingly, the latter can be employed as a basis of classification in such instances, even though the farinograms do not tell the entire story.

"Optimal mixing time" must be regarded as a relative term, depending upon such factors as the type of mixer employed, nature of fermentation, and chemical treatment accorded the dough. It is not a definite or absolute value, except as thus qualified.

American bread flours are, in general, not highly sensitive to mixing treatments, although borderline cases must be recognized, and several such types have been discussed which can be identified by their farinograms and extensograms.

Two types of flours which yield doughs responsive to molding treatment (*a*) the "bucky," overelastic dough and (*b*) the "dead" type of dough which is notably deficient in elasticity, may be distinguished by the area and shape ( $F/E$  ratio) of the extensograms. Strong flours from hard wheats respond more positively to vigorous punching treatment such as is accorded by the make-up machines of the mechanized bakery, or by the dough-forming appliances of the extensograph, than doughs made from softer wheats.

### Literature Cited

- Bohn, L. J., and Bailey, C. H.  
1936a Elasticity of wheat flour dough. *Cereal Chem.* 13: 389-409.  
1936b Effect of mixing on the physical properties of dough. *Cereal Chem.* 13: 560-575.  
Halton, P., and Scott Blair, G. W.  
1936a A study of some physical properties of flour doughs in relation to their bread making qualities. *Cereal Chem.* 14: 201-219.  
1936b A study of some physical properties of flour doughs in relation to their bread-making qualities. *J. Phys. Chem.* 40: 561-580.

## REVIEW OF PROGRESS IN RESEARCH ON BREAD STALING

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(Received for publication September 13, 1939)

The loss due to staling of bread is a serious burden to bakers generally and the economics of bread staling have been discussed by many authors. Almost every baker is aware of the economic side of this problem. Accordingly, the economics of bread staling will be dismissed and the scientific side of the problem considered.

From the technical standpoint losses due to stales can be reduced by producing bread of high quality and long keeping quality, *i.e.*, by keeping bread fresh from time of production to time of consumption. It is probable that all great developments in the future in retarding bread staling will be due to exacting scientific investigation. Scattered practical experiments will help but will do little to clarify this vast complex problem. A great deal of research has already been performed in this field; however, in comparison to what needs to be done, research is only in its beginning.

### Changes that Take Place during Bread Staling

Bread staling processes can be divided into: (1) staling of the crust, and (2) staling of the crumb. The staling of the crust is quite different from the staling of the crumb of the bread. The dry crisp crust of fresh bread becomes soft and leathery as bread stales. The baker increases the rate of staling of the crust by wrapping bread in "moisture-proof" paper. However, this is necessary in order to keep the bread in a sanitary condition.

The process of crust staling is quite easily understood. When fresh bread comes out of the oven the crust is dry and brittle, and as bread ages the moisture from the center of the loaf penetrates to the crust, making it soft and leathery. When bread is wrapped in "moisture-proof" paper, evaporation of moisture from the crust is stopped almost entirely and thus it takes up the moisture from the center of

the loaf, is unable to lose it to the surrounding atmosphere, and becomes soft and leathery more quickly than if it were not wrapped. Unwrapped bread crust will become stale by the same process when the atmosphere is very humid. If the humidity is great enough, the crust may even take up moisture from the air.

Alsberg (1936) defines crumb staleness as "... the change in flavor and texture that develops in time." He considers the word flavor to involve both taste and smell. The texture becomes harder, tougher, and more crumbly as bread stales. The staling of the crumb is a very complex process. Besides the changes mentioned above, the crumb loses its water. However, the above changes (becoming hard and crumbly) occur before the crumb has lost much of its water. Alsberg summarizes the order of these processes as follows: "... first it becomes tougher and harder, next it becomes crumbly, and finally after a much longer time it dries out."

Besides all of these changes, the crumb loses some of its power to swell when placed in water. The crumb of fresh bread swells more than stale bread. This phenomenon was first reported by Lehmann (1894). Also, Lindet (1902) has pointed out that the amount of soluble starch decreases as bread stales. This refers to the amount of starch in the bread that is soluble in water.

These changes on staling occur with whole-wheat and rye bread, as well as with white bread, but to a smaller degree (Katz, 1935). Katz (1928) has stated that they do not occur in baked products of low moisture content, such as zwieback. Breads with little or no starch, such as gluten bread, show these changes only slightly (Katz, 1935).

Summarizing the changes that take place as bread stales, we have: (1) crust becomes soft and leathery, (2) crumb becomes tough and hard, (3) crumb becomes crumbly, (4) crumb loses power to swell in water, (5) amount of soluble starch in crumb decreases, and (6) crumb dries out (*i.e.*, loss of water by evaporation).

An approximate chemical analysis shows little appreciable difference between fresh and stale bread (Cathcart, 1938). Karacsonyi (1928) found that the acidity either remains constant or shows some decrease as bread stales. However, Barnard and Bishop (1914) found that acidity of perfectly stale bread can increase during further keeping, due to the activity of microorganisms. There also are indications of changes in the composition of the fat in very old bread.

### Theory of Crumb Staling

The first ideas of bread crumb staling were that it was due entirely to the loss of moisture. However, as early as 1852 Boussingault



(1874) showed that bread would stale when kept in a container where it cannot lose any moisture. Boussingault then took the bread which had staled without losing any water and made it fresh by heating at 60°C. or higher.

Von Bibra (1861) confirmed Boussingault's findings and extended his observations to include rye bread. Von Bibra also showed that bread containing less than 30% moisture could not be freshened by heating unless it was moistened first. Alsberg (1936) states, "In this connection, it is perhaps significant that native wheat-starch granules when fully hydrated contain about 30% (according to Rodewald, 36%) of water of hydration."

Von Bibra suggests that the moisture in fresh bread is mainly in an uncombined state; however, on staling it enters into chemical combination. Heating supposedly reverses the process and sets the water free; thus the bread is returned to its fresh form if sufficient water is present.

Horsford (1876) suggested an explanation of the process that occurs during staling. He explained that the gluten is dehydrated during baking, while the starch retains most of the water. Thus, during aging the gluten takes up water from the starch. This leaves horny, hard starch particles and accounts for hardness and crumbliness that develop during staling. On reheating the water passes back to the starch from the gluten and the process can be repeated innumerable times.

Boutroux (1897) assumed that the hard, horny starch which is left after the loss of water to the gluten is "a derivative of starch." Lindet (1902) suggested that this "derivative of starch" was simply a less soluble form of starch produced from the starch of fresh bread.<sup>1</sup> This change in form of starch is called retrogradation<sup>2</sup> (the use of this term is questioned by some workers) of the starch and is accompanied by the setting free of moisture to the other components of the loaf because of this change and not because the gluten takes the moisture away from it. This process is more rapid than the development of crumbliness. Katz (1928) explains the delay in the development of crumbliness by assuming that it takes time for water to diffuse from the starch to the protein and of course crumbliness is not noticeable until this diffusion has taken place. This delay in the onset of crumbliness is verified by microscopic examination (Verschaffelt and van Teutem, 1915).

Ostwald (1915) reports that the starch gel of fresh bread<sup>3</sup> is like

<sup>1</sup> Alsberg (1936) points out that the soluble starch of fresh bread is  $\beta$ -amylase.

<sup>2</sup> Lindet called the process retrogradation because he found that the amount of soluble starch decreased as bread staled. However, Lindet also concluded that the gelatinized starch granules imbibe less and less water as the bread stales.

<sup>3</sup> Only first-degree gelatinization.

other gels and that the staling process is simply due to syneresis, *i.e.*, extrusion of water. Some object to this on the basis "that a gel as concentrated as first-degree gelatinized starch does not extrude water."

Katz (1928) has followed the process of staling by the following methods: (1) increase in crumbliness and hardness, (2) decrease in swelling of crumb in water, (3) decrease in amount of soluble starch, and (4) change in X-ray pattern.

On the basis of his results, Katz has explained bread staling as follows: There is a physico-chemical equilibrium set up between the fresh state and stale state. At temperatures of from 60°C. up, the fresh state is the stable form. Thus at temperatures above 60°C.<sup>4</sup> bread will freshen up. At temperatures below 50°C. bread will stale and the lower the temperature the faster it will stale. The maximum rate of staling occurs at about -3°C. By lowering the temperature below this point the rate is decreased again because the bread freezes solid (all reactions are retarded in the solid state). Katz states that at about -193°C. bread will remain fresh indefinitely.

Katz (1928) has shown that the changes which take place in bread staling are very similar to those that take place in a starch gel on standing, and by X-ray studies he has shown that there was the same change in the starch in both cases. Because of this and the fact that bread contains many times (about five or six times) as much starch as protein, the changes in the starch can be taken as the same changes that take place in the staling of bread.

After further work, Katz (1930) modified the above view and states that the process is much more complex and involves a heterogeneous equilibrium. Recent work with X-ray diagrams indicates that in fresh bread the starch exists in an amorphous and crystalline form, while in stale bread it exists totally in a crystalline form (Katz, 1937).

Alsberg (1936) has pointed out that it is possible to explain the changes that take place when bread stales without assuming any chemical change in the starch, any difference between a starch gel and other colloid gels, or without being inconsistent with the known facts of retrogradation. According to Alsberg the staling process is simply an expected physical change of the starch-gluten complex involving the loss of water from both the starch and gluten. He shows that there is no reason to expect the gluten to take up moisture from the starch during the process.

Kuhlmann and Golossowa (1936) have shown that the water-binding capacity of bread crumb gradually decreases during staling and that the water-binding capacity of bread and dough depends upon the method of bread making.

<sup>4</sup> C. H. F. Fuller (1938) finds that bread stales slowly at 60°C. There is definitely a tightening of the crumb at 60° to 100°C., he states.

After reviewing the theories and data (with additional data of his own) of the above authors, Fuller (1938) suggests that during staling gelatinized starch undergoes a reduction in hydration capacity (Kuhlmann and Golossowa, 1936) and an alteration of the proportion of  $\alpha$ - and  $\beta$ -amylose. Fuller explains that there appears to be a definite equilibrium between  $\alpha$ - and  $\beta$ -amylose at any one temperature. He points out that staling can be affected by keeping the proportion of  $\alpha$ - and  $\beta$ -amylose shifted toward the fresh state or by changing the state of hydration of the starch. Heat alters the former, he says; freezing and sugars, the latter.

These are all very important considerations, for, as Alsberg points out, if this change in bread on staling involves a chemical equilibrium it should be possible to find a catalyst which would shift the equilibrium so that staling would not take place at room temperature. If it is not a chemical equilibrium then there is no need to spend time and money searching for a substance (catalyst) that will prevent staling. *Thus, it is evident that it is necessary first to determine the nature of the staling process before looking for substances that will prevent it.* Moreover, it is evident that this theoretical research is of great practical importance. Certain important facts have been established. Much more is to be learned.

### Methods of Measuring Rate of Staling of the Crumb

The methods of measuring staleness have been enumerated by various writers; Alsberg (1936) and Hutchinson (1936) have summarized the methods. They will be extended and summarized here and those which have met with greatest favor given in detail.

*Crumbliness of the crumb.*—Crumbliness cannot be measured accurately. The only method to determine crumbliness is by use of the finger.

*Hardness or compressibility of the crumb.*—Hardness or compressibility of the crumb can be measured quite accurately. This is a method which the baker or bakery technician could very well use in his shop. Methods of measuring compressibility have been described by Bailey (1930), Katz (1917b, 1928, 1934a), and Platt (1930). All of these pieces of apparatus operate on the same principle. An apparatus constructed according to Platt's ideas and used here at the Institute is shown in Figure 1. The apparatus is essentially a large balance with a plunger (A) attached to underside of the right-hand pan. A uniform piece of bread crumb ( $1\frac{1}{2}$  inches thick) is placed on the disk (B). A weight (about 200 g.) is placed on the right pan and a chain weighing exactly the same on the left pan. A 2-gram weight is then added to the right pan; this is just enough extra weight to hold the

plunger down lightly on the bread and steady the pointer (C) so that an initial reading can be taken on the scale (D). Then the chain is removed slowly by means of a string run over a pulley, suspended overhead, and then attached to a windlass (E) equipped with a crank. Sixty seconds after beginning to remove the chain a second reading is taken. The difference between the two readings gives the compressibility of the crumb. In this way compressibility readings can be made as the bread ages. It might be emphasized that as the bread

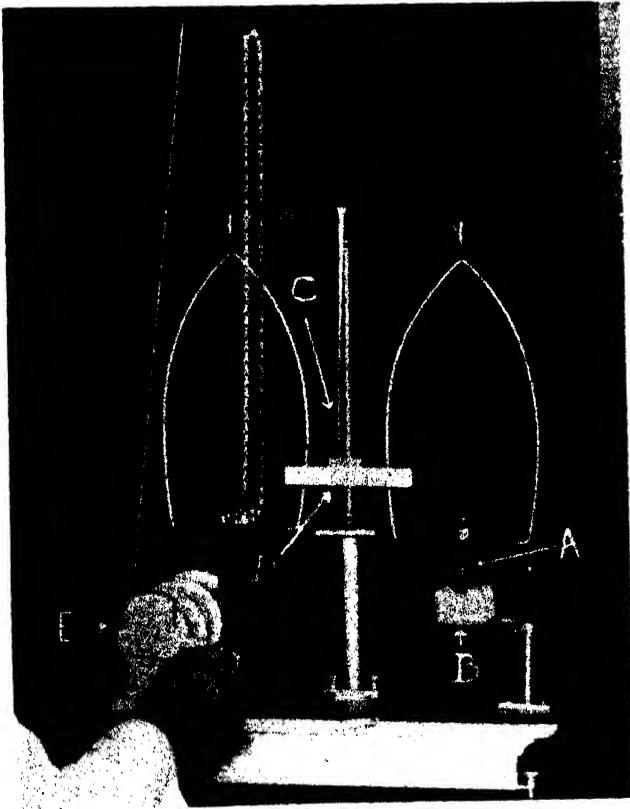


Fig. 1. Apparatus for measuring the compressibility of the crumb of bread.

grows harder the compressibility readings become less. Temperature has considerable effect on these readings so that it should be kept constant over a series of measurements (Platt, 1930).

*Swelling of crumb in water.*—Based on observations of Balland, Lindet, Lehmann and others, Katz (1917b, 1928) has used the following method, based on the swelling of crumb in water, for measuring staleness.

"Ten grams of bread crumb, together with an excess of water, are passed through fine bolting cloth (80 mesh per centimeter). The

volume of the liquid (which is saturated with toluene in order to prevent fermentation) is raised to 250 cc. capacity. After 24 hours the volume of the decantate or deposit is read off. Upon shaking again, another 24 hours is allowed for settling, a second reading made, and the mean of the two readings taken. The volume of the decantate is shown to be considerably larger for fresh bread than for stale bread, for instance 52 cc. compared with 34 cc."

Cathcart and Lubert (1939a) have found that it is much easier to put the bread crumb through a brass-frame, 200-mesh sieve of 5 inches



Fig. 2. Showing how bread crumb is rubbed through sieve. Apparatus, left to right: 250-cc. graduated cylinder, 30-cc. centrifuge tube, 2-liter beaker, sieve on beaker, wash bottle.

in diameter, than the bolting cloth. The sieve has the additional advantage of fitting snugly on top of a 2-liter pyrex beaker, which serves to catch the washings. The apparatus needed is shown in Figure 2. The crumb is rubbed through with the forefinger and the second finger (Fig. 2). A typical example of the difference in the amount of sediment between fresh and stale bread is shown in Figure 3—35 cc. for stale bread and 48 cc. for fresh bread. This test can be applied in the bakery. For rate of staling curves by this method, see Cathcart and Lubert (1939a).

This method has been modified still further by Cathcart and Lubert. Simply, instead of waiting on the sediment to settle for 24 hours, 30 cc. of the sediment suspension is transferred to a centrifuge tube (see Fig. 2) and centrifuged for 2 minutes. The results in cubic centimeters of sediment are read off directly. A typical example of the difference between fresh and stale bread is shown in Figure 4. The difference is less here than with the graduate cylinders because less of the sediment suspension is used. For rate of staling curves by this method see Cathcart and Lubert (1939a).

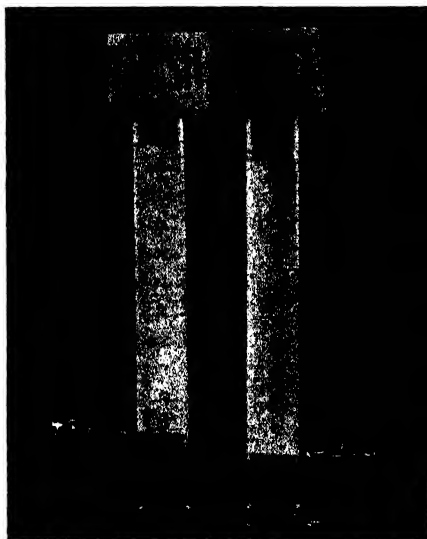


Fig. 3. Showing difference in sediment between fresh and stale bread in graduated cylinders.



Fig. 4. Showing difference in sediment between fresh and stale bread in centrifuge tubes.

Karacsonyi (1929) has also modified the above method. Instead of letting the sediment suspension stand 24 hours he measures the viscosity of it. It is reported that as the bread ages the viscosity decreases.

Fuller (1938) has modified this method as follows: he determines the amount of water to give a standard consistency in a farinograph with a definite amount of shredded bread crumb. The main objection is that bread made with diastatic malt extract or a similar substance gives low values (about the same as stale bread) when fresh and the values fail to decrease on staling. Fuller did find, however, that starch gels showed a decrease as did bread (except that made with diastatic malt), which agrees with the results of Katz's X-ray studies, that the starch is the important component involved when bread stales.

*Amount of soluble starch.*—This method is complex and time-consuming. However, it is interesting to know that such a method exists

and that the amount of soluble starch decreases as bread stales. Katz (1928) describes the method in detail and considers this method less satisfactory than the one based on the swelling of the crumb in water (Katz, 1917a, 1928).

*Opacity of crumb.*—Glabau and Goldman (1938) have found that the starch gel of bread becomes more opaque as the bread ages. By means of a photoelectric cell "set-up" the light that passed through the bread under definite conditions was measured. The change in opacity parallels the change in crumbliness. This method of measuring staleness is not a modification of the "swelling of crumb in water" method; however, both are undoubtedly due to the same change in the starch.

*X-ray pattern.*—X-ray studies of bread have been made, notably by Katz (1928, 1930, 1937). Although X-ray does not offer a method of following the rate of staling, it has definitely shown, as mentioned above, that the starch is in a different form in stale bread than it is in the fresh product. Thus, from a technical standpoint staling is simply due to this change in the starch. This change in the form of starch seems to account for the decrease in compressibility of the crumb, decrease in the swelling of the crumb in water, and decrease in amount of soluble starch.

*Loss of water by evaporation.*—The loss of water by evaporation has been mentioned above as the last step in the staling process and can be accurately measured by laboratory methods. However, as pointed out above it does not bear much relationship to the changes in the starch, since the change in the starch will take place even if evaporation is prevented. Nevertheless, in commercial practice today, the loss of moisture is of the utmost importance. For further consideration of this factor, please see page 116, "Physical Methods vs. Human Beings for Determining Staleness."

### Retarding the Staling of the Crust

Crust staling can be prevented by proper storage methods. When bread is wrapped (crumb at approximately 85°F.) moisture passes from the inside crumb to the crust. Thus the crust becomes soft, for it cannot lose water to its surroundings because of the moisture-proof wrapper. In general, any method of preventing the crumb from losing water increases the staling of the crust. Crust staling can be prevented by storage in a moderately dry place after adequate cooling.

Katz has patented a process<sup>5</sup> for preventing crust staling. This consists mainly of a chamber in which unwrapped bread can be kept

<sup>5</sup> This process was used to a small extent in Holland to keep bread over night.

in a circulating atmosphere of from 65% to 70% relative humidity. The upper limit prevents the crust from taking up water and the lower limit prevents the bread from drying out too rapidly.

If bread were allowed to dry out more than is the usual custom before it is wrapped, the crust would keep better; however, this would cause the crumb to be more dry than is desirable. Thus, the crust is sacrificed for the crumb.

Barnard and Bishop (1914) have shown that if bread is allowed to cool in a fairly dry atmosphere and storage temperature before wrapping, the crust does not absorb water from the crumb. They believe that most of the fault comes from wrapping the bread too warm. The modern rapid cooling methods offer means of cooling bread to the proper temperature without an excessive loss of moisture. This subject has been gone into quite thoroughly by Berg (1926).

Hutchinson (1936) lists the following as the chief factors in the production of crisp crust of long life: (1) flour type, (2) fermentation procedure, (3) method, time, temperature of baking, and (4) storage conditions. He points out that very little is known about the first two, and that the last two are the most important. In regard to the third point Hutchinson says: "Undoubtedly well-baked crusty bread does seem to stale *in the crust* more rapidly than unburnt crust, and the leathery tough nature of most crusts seems to be due to the method of production of the crust quite as much as to storage conditions." Steam in the oven is cited as an example of the latter. That is, the crust of bread baked in an oven with steam frequently becomes very tough as compared to that baked without steam. The one with steam, however, has a much better crust color and bloom.

Alsberg (1936) considers that the following will produce a crust that will remain crisp for a long time: (1) high fermentation temperature, (2) a high proportion of yeast, (3) as little water as practicable in dough, and (4) small amount of salt. From the next section it will be noted that none of these factors is beneficial as far as crumb staling is concerned.

Shortening has a beneficial effect on the crust. Hutchinson states, "It is known that the influence of shortening agents upon the crust is very marked, frequently yielding crust of improved palatableness, crispness, and general attractiveness."

These factors which affect the crust are summarized briefly in Table I.

### **Retarding the Staling of the Crumb**

The crumb staling process may be retarded by various methods. The methods which demand attention are heat, refrigeration, wrap-



TABLE I  
EFFECT OF VARIOUS FACTORS ON THE RATE OF STALING OF BREAD CRUST

Factors which affect crust staling	Effect on rate of crust staling	Reported by
Flour type	Best type not determined	Hutchinson
Fermentation procedure	Best procedure not determined	Hutchinson
Method of baking	Best method not reported	Hutchinson
Time of baking on short side	Decreases rate	Hutchinson
Temperature of baking	Best temp. not reported	Hutchinson
Steam in oven	Increases rate	Generally known and Hutchinson
High fermentation temperature	Decreases rate	Alsberg
High amount of yeast	Decreases rate	Alsberg
Little water as practicable in dough	Decreases rate	Alsberg
Small amount of salt	Decreases rate	Alsberg
Shortening	Decreases rate	Hutchinson
Moderately dry storage	Decreases rate	Katz
Wrapping	Increases rate	Generally known
Proper wrapping	Neither increases nor decreases rate	Barnard and Bishop

ping, ingredients, and methods of manufacture. The baking industry is fairly well agreed that certain methods of manufacture, etc. (see below) will prolong the life of bread. Katz (1917a) and Alsberg (1936) state that the test for staling based on the swelling of the crumb in water does not show that any of these procedures of manufacture delay the aging of the starch granules of the crumb. Not enough work has been done on the method based on the compressibility of the crumb or others to draw conclusions as to how results from them compare with the swelling-power method. Steller and Bailey (1938) found that the compressibility and viscosity methods (noted under method based on swelling of crumb in water above) gave results that were more consistent and uniform than the data obtained by swelling of the crumb in water. They also point out that the latter method is not sensitive to minor changes in the condition of the bread. The writer has found that this property is also true in regard to temperature of the bread and room. Thus, in this respect, the latter method is more desirable, for variations in temperature will cause less variation in the results obtained.

As Alsberg (1936) has pointed out, "It cannot be doubted that some of these practical procedures do have the effect claimed for them." The explanation probably is that the method based on the swelling of crumb in water tells the state of the starch but that there are other things that must be taken into consideration. For example, shortening is known to lengthen the life of bread. This is not due to the delay in the changes in the starch but probably to the way it masks the effects of these changes.

The following are various methods of prolonging the life of the crumb of bread:

*Heat.*—The reheating of bread to freshen it has been known for a long time. The experiments of Boussingault (1874) have been mentioned before. Katz (1928) and others have done a great deal of work on this method. It can be concluded that holding bread at 60°C. or more will maintain it in a fresh condition. Many investigators consider that bread will remain fresh indefinitely at 60°C.; however, Fuller (1938) has reported that staling occurs slowly even at this temperature. The chief objection to this procedure is that bacteria generally develop inside the crumb, producing a penetrating off-aroma. Katz states that this aroma is generally detectable after 12 to 24 hours. With addition of acid salts to bread, the heating method has been applied for periods of about 12 hours. It is important that ventilation and humidity be controlled so that the crust of the loaves will not become soft and leathery or become unduly dry. On the whole, this method does not seem to offer practical possibilities due to development of the penetrating off-aroma and due to the crusty flavor which is generally imparted to the crumb.

*Refrigeration.*—As pointed out before, the maximum rate of staling of bread occurs at about  $-3^{\circ}\text{C}$ . However, as Katz (1928) and others report, temperatures of about  $-10^{\circ}\text{C}$ . retard the staling process greatly. Alsberg (1936) even says that temperatures of from  $-10^{\circ}\text{C}$ . to  $-20^{\circ}\text{C}$ . prevent it altogether. Because of the many conflicting reports, and in order to determine just how long bread can be kept fresh at freezing temperatures, Cathcart and Lubber (1939b) have investigated the problem. They have found that at temperatures of  $-22^{\circ}\text{C}$ . bread can be maintained fresh for approximately 30 days according to scientific scoring methods (according to the swelling-power test it was practically stale in 24 hours) and at  $-35^{\circ}\text{C}$ . for approximately 70 days. In the latter case it was also fairly fresh according to the changes in the starch. The bread was handled in a commercial manner throughout and the crust was kept in good condition by keeping the bread wrapped in moisture-proof wrappers during freezing and thawing. The method is very promising and might prove practical in helping the baker get bread to the consumer in a fresher condition. It would, of course, be of great help in emergencies. As a result of the development of refrigerated warehouses, it seems to be the most promising method of the future.

*Wrapping.*—Bread is wrapped to keep it in a sanitary condition and to minimize the drying out as much as possible. Wrapping delays staling only insofar as the loss of moisture is connected with staling. As pointed out above, the change in the starch takes place even though

the loss of moisture is prevented. Thus, the fact that a loaf of bread has not lost much of its moisture does not mean that it is fresh as far as the starch is concerned. Nevertheless, wrapping plays a considerable role as far as the consumer is concerned by decreasing drying-out and at the same time loss in weight. Thus, from this latter standpoint, wrapping increases the life of bread.

Wrapping so retards the loss of moisture that the usual hard, dry crumb just underneath the crust, which always becomes evident in 24 hours with unwrapped bread, never develops. As mentioned before it allows the moisture in bread to equalize itself between the interior crumb and the crust.

It is interesting to note that Platt (1930) found little difference between the compressibility of central crumb of wrapped and unwrapped bread on aging.

Morison and Gerber (1925) and also Barnard (1924) give the moisture content of unwrapped bread as it dries out in the ordinary atmosphere. There is a rapid fall in moisture content in the first 48 hours. Results by Cathcart and Pushnik (1939) on sliced, moisture-proof wrapped bread show that from the time of wrapping until the bread is 72 hours old, only about a 2% loss of moisture occurs.

*Ingredients.*—Hutchinson (1936) has divided substances that have been recommended for addition to bread dough to retard staling into two classes: (1) those that prevent or delay the change in the starch and (2) those that improve bread quality. Substances belonging to the first class would be of utmost commercial value. However, as Hutchinson and also Alsberg (1936) have stated, a substance which will do this without detracting from the good qualities of normal bread has not been found. It should be mentioned, however, that Katz (1917a, 1917b, 1928) found that aldehydes and alkaline bases would do it, but they have *undesirable* physiological effects and large quantities are required; thus are of no commercial value.

Substances belonging to the second class are those that help to give better bread from the beginning or act as moisture retainers. These substances offset the changes in the starch but only delay this change slightly if any at all. These substances may be listed as follows: flour (wheat and rye), yeast, milk, salt, gelatinized starch (scalded flours, dextrinized flours, dextrin, potato flours, etc.), shortening, malt extract, sour dough, protective colloids (agar agar, mayonnaise, lecithin, etc.), gelatin, glucose (corn sugar), glycerin, soy flour, whey, egg whites, etc.

All workers<sup>6</sup> agree that flour is very important and that a flour

<sup>6</sup> For references to Alsberg and Hutchinson in this and the following sections, see Alsberg (1936) and Hutchinson (1936).

containing a large amount of high-quality gluten, if properly fermented, will prolong the life of bread. Alsberg states that the degree of refining of flour seems to have some influence and that very fine grinding is unfavorable. It is important to remember as Hutchinson states that "the staling problem is at a minimum with the best possible loaf made from the best possible flour." Dearsley (1925) has stressed the advantage of good-quality gluten. Katz (1934b) has also pointed out the importance of using high-grade flour. Jago and Jago (1911) state that some flours, particularly those with a high percentage of protein, readily become somewhat dry and harsh.

Alsberg reports that 5% to 10% of rye flour will slightly retard staling. This agrees with the accepted fact that rye bread does not stale as rapidly as white bread. Figures 3 and 4 of Cathcart and Lubert's article (1939a) illustrate this latter fact. The two top curves in each figure show the centrifuge modification of the swelling-of-crumb-in-water method. The bottom curves show the regular Katz swelling-of-the-crumb-in-water method. It will be noted that the curves of the figure for white bread show a faster lowering of the degree of swelling than the curves for rye bread.

Alsberg says that the use of "not too much yeast" is favorable. He reports that yeast quality is important; however, he says that different types of yeast have not yet been adequately investigated. Alsberg also reports that a normal amount of salt is favorable.

Both Alsberg and Hutchinson agree that milk, shortening, malt extract, and gelatinized starch are favorable. The use of milk has also been stressed by Davis and Eldred (1923) and the American Institute of Baking.<sup>7</sup> The work of Glabau and Goldman (1938) also indicates that milk is favorable. Katz (1934b) also says that shortening, milk, and not too much yeast, are favorable to long life.

Bailey (1932) found that potato flour, malt extract, sour dough, and agar agar had a slight beneficial effect in reducing hardness by compressibility. Mayonnaise and two vegetable lecithins had no effect. Hutchinson says that his own observations agree with those of Bailey and states that the reduction of hardness is "probably a case of softer loaves initially rather than a real retardation of hardness development." Bailey also tried dextrinized starch, various sugars,<sup>8</sup> a great variety of dairy products and calcium peroxide (increases absorption). None had effect on the compressibility or the degree-

<sup>7</sup> Annual report, 1924.

<sup>8</sup> In contrast to this A. G. Kul'man and E. P. Balasheva (Tekhnol. Protssessy i Kontrol Pishchevoi Ind., 175-198, 1938) state that sugars retard the rate of staling. Listing carbohydrates in order with the most effective first they gave maltose syrup, glucose syrup, dextrin, beet sugar, maltose, glucose, soluble starch, and potato flour. Soluble starch and potato flour gave bread which turned stale sooner than bread without any special addition. Also A. K. Epstein and R. R. Harris (Baker's Helper 64: 347, 1935) have patented the use of arabinose to retard staling.

of-swelling-of-crumb test. Gelatinized starch was found to be favorable by Katz (1934b).

In addition to the above, Hutchinson says that it is believed that gelatin, glucose, and glycerin prevent staling by retaining moisture.<sup>9</sup> Glycerin is now generally discredited for this purpose. As long ago as 1911, Jago and Jago (1911) listed gelatinized starch, dextrin, boiled potatoes, and potato flour as keeping bread moist longer. Also Whympers (1919) listed glycerin, glucose, malt extract, scalded potatoes, and potato flour as moisture retainers. Hutchinson classed soy flour and whey as slightly favorable. Steller and Bailey (1938) have concluded that the use of 1½% of free-fat soy flour reduced the rate of staling (change in starch) of bread, yet from a review of their data one is forced to conclude that the effect is very slight. Kirkland (reported by Hutchinson) performed many experiments on some of the above-mentioned substances and his results agree favorably with the reports of Alsberg and Hutchinson.

Banfield (1938) states that egg whites (six egg whites to 14 lbs. of meal) in a dough from which brown bread is made will produce brown bread which will keep moist for 36 hours longer than ordinary brown loaves. Egg yolk which contains lecithin is stated as being an excellent stabilizer for unstable doughs, but its effect on staling is not mentioned. Banfield says that potato mash and rye flour added to white dough favor moist, good-keeping bread. Gelatinized starches, scalded flour, gelatin, and agar agar give only a false water absorption according to Banfield; although they can hold much water in the cold, they will break down in the oven.

Hutchinson stresses a point that is very important to remember. That is, "many of the substances recommended for prevention of rapid staling have some undesirable effect which may counterbalance any good effect on staling; thus glycerol delays fermentation, and underfermentation invariably leads to rapid staling of bread." Soy flour is another example pointed out by Hutchinson; no real gain results from using it, for in quantities above 1½% to 2% of the weight of the flour there is a reduction in loaf volume, a deterioration in texture, and the development of an undesirable crumb color. Many other substances belong to this class; that is, agar agar, casein, and the like. However, not all of the substances listed above belong to this class.

In general, bread from a rich formula is preferable; that is, has better keeping qualities from the practical standpoint than bread from a lean formula. Platt (1930) has shown that rich-formula bread has greater compressibility.

<sup>9</sup> Treatment of dough with infra-red rays (Baker's Helper 61: 533, 1934) is said to retard staling by increasing the moisture content.

*Methods of manufacture.*—Besides the factors mentioned above, which affect the rate of staling of bread, certain methods of manufacture are said to be favorable. The following are those to be considered: absorption, mixing, time and temperature of fermentation, type of fermentation, handling, baking, and cooling.

Hutchinson believes that a slack dough is favorable to a longer life of bread. Alsberg states that high-speed mixing will prolong the life of bread and Hutchinson adds that the dough should not be over- or undermixed. According to Alsberg and Katz (1934b) a long fermentation at low temperature is favorable. Underfermentation is one of the chief causes of poor keeping quality, Hutchinson writes. Of course the important thing is *proper* fermentation. Proper fermentation is probably of more importance than any one other single factor with the exception of heat and refrigeration.

Sponge fermentation is preferable to straight-dough fermentation according to Alsberg. The long-sponge system is recommended in preference to the shorter straight-dough system by Kent-Jones (1927). Of course, sour-dough fermentation is advantageous as mentioned above. Hutchinson states that in his work short-time processes with ordinary bread-making flours frequently gave bread of better keeping quality than that made by long-straight and sponge-dough systems. The important factor seems to be the correct adjustment of fermentation period to the flour and other ingredients. Hutchinson states: "though many bakers believe that bread made with large quantities of yeast at relatively high dough temperatures has poor keeping quality, we cannot agree, though undoubtedly the risk of incorrect fermentation is considerably enhanced when very short processes, *e.g.*, at relatively high temperatures, are employed. At present, the most important method of combating loss of keeping quality is to produce the best loaf of which really good flour is capable. To state that a certain method of fermentation gives bread of better keeping quality than another system is of little significance unless all of the conditions, type of flour, etc., be stressed, and those keeping qualities considered to be of primary importance, specified."

Both Alsberg and Hutchinson agree that the proper handling of a dough is important. One should be careful to punch at optimum time and not permit overmanipulation in machine-made doughs. Alsberg and Katz (1934b) state that baking should be carried out slowly in an oven that is not too hot; overbaking is to be avoided. Hutchinson stresses that one should be careful not to underbake bread. One would take these statements to mean that the important thing is proper baking; however, none of the above authors states just what

proper baking is. Katz stressed the art and skill that a baker must possess and its important part in producing a loaf of long life.

Alsberg says that the loaf should not lose too much moisture in cooling. He recommends rapid cooling, provided too much moisture is not lost by so doing. Alsberg summarizes the factors which prevent the aging of the crumb of the bread as follows:

- "1. Anything that increases the water content seems to be favorable to long life. . . .
- "2. Anything that hampers the mobility of moisture (prevents evaporation) in the cooled loaf perhaps prolongs life. . . .
- "3. Anything that conceals the effects of aging of starch prolongs the loaf's life in that the loaf remains acceptable to the consumer. . . ."

All the factors listed and discussed under this heading are summarized in Table II.

#### **Physical (Mechanical) Methods vs. Human Beings for Determining Staleness**

There are two ways of measuring staleness: (1) the physical or mechanical means which were mentioned under "Methods of Measuring Rate of Staling of Bread Crumb" and (2) examination by human beings. The factor which greatly controls the opinion of human beings is the moisture content. And in general any one of the factors listed in Table II which increases the moisture content and favors its retention will increase the life of bread as far as human beings are concerned. From tests performed (Cathcart and Lubert, 1939b) factors of freshness which human beings consider to be very important are not necessarily ones which the physical tests measure (*i.e.*, changes in the starch). Now which is of the more importance, physical tests or those by human beings?

This question might be answered simply by saying that tests by people are not scientific, while the mechanical tests are. And, since scientific tests are superior to unscientific human-being tests, the scientific tests are to be preferred. Both Platt (1930) and Alsberg (1936) have pointed out how unreliable such judgments of people may be. Thus, on the basis of tests by human beings "it is idle to speculate about the reasons why specific procedures or specific ingredients act as they do." Yet human consumers buy the bread.

The physical tests are important in that they give us definite numerical results, which are unbiased and fairly reproducible. Much time and effort have been spent in developing these physical tests; however, in general they show (on basis of work so far) that the

TABLE II  
EFFECT OF VARIOUS FACTORS ON THE RATE OF STALING OF BREAD CRUMB

Factor	Effect on the		Reported by
	Rate of practical staling	Rate of change of starch	
Heat, 60° C.	Decreases rate	Decreases rate	Katz, others
Refrigeration, -22° C.	Decreases rate	Decreases rate	Cathcart and Luber
Refrigeration, -35° C.	Greatly decreases	Greatly decreases	Cathcart and Luber
Wrapping	Decreases rate	No effect	Boussingault, Katz, others
Flour (large % and high-quality gluten)	Decreases rate	No effect	Alsberg, Hutchinson, Katz, others
Rye flour (5%-10%)	Slightly decreases	Little effect	Alsberg, Banfield
Yeast	?	No effect	Alsberg
Milk	Decreases rate	No effect	Alsberg, Hutchinson, Katz, others
Normal % salt	Decreases rate	No effect	Alsberg
Gelatinized starch, etc.	Slightly decreases rate	No effect	Alsberg, Hutchinson, Bailey, Katz, Jago and Jago, Whymp
Shortening	Decreases rate	No effect	Alsberg, Hutchinson, Katz
Malt extract	Slightly decreases	No effect	Alsberg, Bailey, Hutchinson, Whymp
Sour dough	Slightly decreases	No effect	Bailey
Protective colloids	Slightly decreases	No effect	Bailey, Hutchinson
Gelatin	Slightly decreases	No effect	Hutchinson
Glucose	Slightly decreases	No effect	Hutchinson, Bailey, Whymp
Maltose and glucose syrup	Slightly decreases	No effect	Kul'man, Balasheva
Invert sugar	Slightly decreases	No effect	Bailey
Glycerin	Slightly decreases	No effect	Bailey, Whymp
Soy flour	Slightly decreases rate	Very slightly decreases rate	Hutchinson, Steller, Bailey
Whey	Slightly decreases	No effect	Hutchinson
Egg whites	Slightly decreases rate	Probably no effect	Banfield
Slack dough	Decreases rate	No effect	Hutchinson
Optimum high-speed mixing	Decreases rate	No effect	Alsberg, Hutchinson
Proper fermentation	Decreases rate	No effect	Alsberg, Hutchinson, Katz
Sponge fermentation	Decreases rate	No effect	Alsberg, Kent-Jones
Long fermentation	Decreases rate	No effect	Kent-Jones, Katz
Proper handling	Slightly decreases rate	No effect	Alsberg, Hutchinson, Katz
Proper baking	Slightly decreases rate	?	Alsberg, Hutchinson, Katz
Proper cooling	Decreases rate	No effect	Alsberg

crumb of bread is stale long before human beings think so. In general the two methods of measuring staling do not agree. This was pointed out above and is evident from Table II.

This does not mean that the physical tests are useless—far from it. They are the only reliable means that are available for following stale-



ness. They will give accurate means of comparing various factors of baking, ingredients, etc., as to their ability to prevent the change in the starch. However, more time and effort are necessary in order to develop physical tests, the results of which will be in better agreement with the thoughts of the consumers—human beings. It might be pointed out that as yet some of the existing methods have not been tested thoroughly enough; more work is needed on them to attempt to correlate the results with the thoughts of people.

The tests made by Cathcart and Lubert on the freezing of bread to retard staling showed that, according to the tests based on the swelling of the starch in water, the bread was stale long before the human judges found it so.

### **Digestibility of Fresh and Stale Bread**

Is fresh bread more digestible than stale bread? This question is often brought to our attention and many who have not actually asked the question have probably pondered over it.

Long ago Jungmann (1895) was able to find little difference, if any, between the action on fresh and on stale bread by the saliva of the mouth and by a ferment similar in part to that found in the stomach (pepsin-hydrochloric acid). Jungmann pointed out, although he was unable to prove it, that fresh bread might cause sensations of distress due to the fact that it forms lumps, since it requires little chewing for swallowing.

In 1904 Roux (1904) took up the study and concluded that stale bread is no more nor no less digestible than fresh bread.

Katz (1928) found that dogs secreted practically the same quantity of saliva, gastric juice, or pancreatic juice when fed fresh and stale bread. He also found that the enzyme diastase more readily attacked the starch of fresh bread than that of stale bread. These facts would indicate that fresh bread is the more digestible. However, Katz was able to show that, after salivation and chewing, stale bread was crumbly, while fresh bread formed heavy lumps. Those heavy lumps, of course, are more difficult to digest, as pointed out by Hammond (1857). Thus fresh bread which has been thoroughly masticated would be just as easily digested, if not better, than stale bread.

Alsberg (1936) suggests that in former times fresh bread was "promoted" as less digestible than stale bread, for relatively stale bread was served to keep down consumption. The serving of stale bread to reduce consumption is practiced by countries threatened with a wheat shortage even today. Alsberg concludes: "Under the conditions of the present, there seem to be no scientific data on record demonstrating any material difference in the food value or wholesomeness of fresh

and of stale bread." Thus stale bread is as nutritious as fresh bread (and *vice versa*) when protected from mold and contact infection.

### Summary and Conclusions

This review explains to the best of our present knowledge what happens when bread stales, discusses methods of measuring staleness, and summarizes methods of preventing staleness. The inadequacy of our present knowledge of what happens when bread stales; the disagreement between physical or mechanical methods and the results of human judges as to the rate of bread staling; the lack of much scientific research on the effect of certain processes, ingredients, etc., in preventing bread staling; and the disagreement of existing data make definite conclusions difficult and point to the need for further strictly controlled scientific researches.

Some processes, ingredients, etc. have an effect on delaying staleness, while others have little effect, if any at all, and in some cases even decrease bread quality.

Many methods recommended for delaying crust staling increase crumb staling and *vice versa*. The two most effective methods for delaying crumb staling are heat and cold, yet these are not entirely practical at the present time. Considering the data presented for retarding the staling of crust and crumb it seems probable that a palatable loaf of good keeping qualities can be produced by:

1. Using flour with high-quality gluten,
2. Using liberal amounts of milk, shortening, and sugar (preferably sirup),
3. Using normal amounts of salt and yeast,
4. Using some malt extract and some moisture-retaining agent such as gelatinized starch,
5. Making a medium-slack dough by high-speed mixing,
6. Proper fermentation, handling, and baking,
7. Rapid cooling and wrapping after adequate cooling.

### Acknowledgment

The author is indebted to several people in the baking and allied industries for reviewing the manuscript.

### Literature Cited

- Alsberg, C. L.  
1936 The stale-bread problem. *Wheat Studies* 12: 221-247.
- Bailey, L. H.  
1930 A simple apparatus for measuring the compressibility of baked products. *Cereal Chem.* 7: 340-341.  
1932 The use of certain constituents in bread making with particular reference to the problem of staling. *Cereal Chem.* 9: 65-70.
- Banfield, W. T.  
1938 Manna. Maclaren and Sons, Ltd., London.

- Barnard, H. E.  
1924 Standard bread weight laws. *Baking Tech.* 3: 110-114.  
— and Bishop, H. E.  
1914 The effect of bread wrapping on the chemical composition of the loaf. *Ind. Eng. Chem.* 6: 736-746.
- Berg, I. A.  
1926 The cooling of bread. *Baking Tech.* 5: 205-208; 246-251; 279-282; and 328-330. See also *A. S. B. E. Bul.* 39.
- Boussingault, J. B.  
1874 *Agronomie, chimie agricole, et physiologie* (2nd ed.), Gauthier-Villars, Paris; see also *Ann. chim. phys.* III, 36: 490 (1853).
- Boutroux, L.  
1897 *Le pain et la panification*, Baillière, Paris.
- Cathcart, W. H.  
1938 Unpublished observations.  
— and Lubber, S. V.  
1939a Modification of the "swelling power" test for the staling of bread. *Cereal Chem.* 16: 430-440.  
1939b Freezing as a means of retarding bread staling. *Ind. Eng. Chem.* 31: 362-368. See also *Food Industries*, Feb., p. 68, and April, p. 200.  
— and Pushnik, W.  
1939 Unpublished observations.
- Davis, J. S., and Eldred, W.  
1923 Stale bread loss as a problem of the baking industry. *Food Res. Inst.*, Publication No. 1.
- Dearsley, W. L. M.  
1925 Chemistry of bread. *New Zealand J. Sci. Tech.* 8: 34.
- Fuller, C. H. F.  
1938 Starch and bread staling. *Chemistry & Industry* 57: 562-568.
- Glabau, C. A., and Goldman, P. F.  
1938 A study of certain physical properties of bread through the staling process with the use of the electronic cell. *Cereal Chem.* 15: 541-551.
- Hammond, W. A.  
1857 Experimental researches relative to the nutritive value and physiological effects of albumen, starch, and gum, when singly and exclusively used as food. *Trans. Am. Med. Association* 10: 511-587.
- Horsford, E. N.  
1876 Report on Vienna bread in "Reports of the Commissioners of the United States to the International Exhibition held at Vienna, 1873," Government Printing Office, Washington, 2: 1-122.
- Hutchinson, J. B.  
1936 The staling and keeping quality of bread. *Research Assoc. British Flour Millers*, Special Report No. 15.
- Jago, W., and Jago, W. C.  
1911 The technology of bread making. Simpkin, Marshall, Hamilton, Kent, and Co., Ltd., London.
- Jungmann, E.  
1895 Studien über Mehl und Brot. *Arch. Hyg.* 24: 109-123.
- Karacsonyi, L. P.  
1928 Staling and hydrogen-ion concentration. *Cereal Chem.* 5: 477-481.  
1929 A quick viscosimetric method for measuring the staleness of bread. *Cereal Chem.* 6: 241-243; see also *Magyar Chem. Folyóirat* 36: 59 (1930).
- Katz, J. R.  
1917a Het oudbakken worden van het brood in verband met het vraagstuk van dem nachtarbeid der bakkers. Dissertation, Amsterdam, 1917; Gravenhage, van Langenhuisen.  
1917b Onderzoekingen naar het oudbakken worden van brood en de middelen van het te voorkomen. Ministerium für Ackerbau. Industrie u. Handel, Holland.  
1928 Gelatinization and retrogradation of starch in relation to the problem of bread staling. Chapter in "A Comprehensive Survey of Starch Chemistry," by R. P. Walton. The Chemical Catalog Co., New York, N. Y., pp. 100-117.  
1930 Abhandlungen zur physikalischen Chemie der Stärke und der Brotbereitung. I. Über die Änderungen im Röntgenspektrum der Stärke

- beim Backen und beim Altbackenwerden des Brotes. *Z. physik. Chem.* **A150**: 37-59.
- 1934a What is the fundamental change in the staling of the bread crumb? *Bakers' Weekly*, Dec. 1, p. 31.
- 1934b Can we retard or prevent the staling of bread? *Bakers' Weekly*, Feb. 10, p. 35.
- 1935 The staling of breads other than white wheat bread. *Bakers' Weekly*, Feb. 2, p. 37.
- 1937 The amorphous part of starch in fresh bread and in fresh pastes and solutions of starch. *Rec. trav. chim.* **56**: 785-793.
- Kent-Jones, S. W.  
1927 *Modern cereal chemistry*. Northern Publishing Co., Ltd., Liverpool.
- Kuhlmann, A. G., and Golossowa, O. N.  
1936 Bound water in bread. *Cereal Chem.* **13**: 202-217.
- Lehmann, K. B.  
1894 Hygienische Studien über Mehl und Brot. Theil V: Beiträge zur physikalischen Beschaffenheit des Brotes. *Arch. Hyg.* **21**: 213-267.
- Lindet, L.  
1902 Sur les états, que présente l'amidon dans le pain tendre et dans le pain rassis. *Bul. Soc. Chim. de Paris* **27**: 634-639.
- Morison, C. B., and Gerber, L. P.  
1925 Note on weight losses of bread. *Baking Tech.* **4**: 304-306.
- Ostwald, W.  
1915 Die Welt der vernachlässigten Dimensionen. Steinkopff, Dresden and Leipzig.
- Platt, W.  
1930 Staling of bread. *Cereal Chem.* **7**: 1-34.
- Roux, E.  
1904 Sur l'état de l'amidon dans le pain rassis. *Compt. rend.* **138**: 1356-1358.
- Steller, W. R., and Bailey, C. H.  
1938 The relation of flour strength, soy flour, and temperature of storage to the staling of bread. *Cereal Chem.* **15**: 391-401.
- Verschaffelt, E., and Van Teutem, E.  
1915 Die Änderung der mikroskopischen Struktur des Brotes beim Altbackenwerden. *Z. physiol. Chem.* **95**: 130-135.
- Von Bibra, E.  
1861 *Getreidearten und das Brot*, Nürnberg.
- Whympier, R.  
1919 The conditions which govern staleness in bread. Maclaren and Sons, Ltd., London.

## DETERMINATION OF AMINO NITROGEN IN MALT EXTRACTS

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(Received for publication July 22, 1939)

The need of a reliable method for the determination of amino nitrogen in malt extracts has been recognized by malt and brewing chemists for some time. Many methods have been proposed yet none is entirely free of inaccuracies.

The methods outlined in *Cereal Laboratory Methods* of the American Association of Cereal Chemists (1935) and *Methods of Analysis* of the Association of Official Agricultural Chemists (1935) are not sufficiently precise to justify their application to the determination of

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amino nitrogen in malt without considerable modification. Methods now in use in the various malt and brewing laboratories are: (a) Van Slyke's gasometric procedure (1911, 1912, 1913, 1929); (b) Foreman's alcoholic titration (1920); (c) Kolbach's modification (1928) of Sørensen's formol titration (1907, 1908), according to Pawlowski (1932); (d) Walters' modification (1937) of Folin's colorimetric method (1922); and some few electrometric titration methods of which the detailed procedure is unknown.

Considerations of primary importance in the selection of a method for routine purposes are simplicity, rapidity, accuracy, and consistency.

According to Richardson (1934), the Van Slyke method (a) is imperfect as it does not accurately estimate glycine, cystine, tryptophane, arginine, lysine and glutamine.

Foreman's alcoholic or acetone titrations (b) are inapplicable to malt extracts, as magnesium salts and various weak acids are titrated as amino nitrogen (Richardson, 1934).

Walters' colorimetric method (d) requires too much time and precision to be of any practical use. Employing the colorimetric, Van Slyke and Sørensen methods, Walters obtained results colorimetrically that compared favorably with those by the Van Slyke method, though considerably lower than those by the Sørensen titration.

The formol titration of Kolbach, outlined by Pawlowski (c), appears to be the most acceptable method, notwithstanding the adverse criticism of contemporary investigators. In defense of this statement it is necessary to consider the controversial aspects of the method.

Schryver and Thomas (1929) object to the lack of a suitable indicator. Walters is in agreement in this respect, maintaining that the end point is masked and that he could not attain the degree of precision claimed by Kolbach. Walters also objects to the cumbersome method of procedure. There is some justification for the latter criticism and for that reason certain deviations from the standard procedure are later proposed by the author. Richardson prefers the Van Slyke method for colored biological extracts even though uncertain results obtain. No alternate procedure is suggested by Richardson for the application of the formol titration for colored extracts that have not been treated with decolorants. By his admission, a titration at 50% neutralization covering a range of 4 pH units permits 97% titration, and a more complete estimation of amino acids with weak  $\text{NH}_2$  groups. The author has experienced very little difficulty in measuring the color of the test solution, except in the instance of extremely dark-colored (caramel malt) extracts. It seems preferable, therefore, to follow the regular procedure in the formol method, rather than use a decolorant

that would remove 15% to 25% amino nitrogen. The color of normal extracts of malt does not interfere with the measurement of color to any serious extent, if properly compensated.

Some of the precautions outlined by Pawlowski were found to be superfluous, *viz*: adjustment of the formaldehyde, preparation of buffer solutions, and addition of barium chloride crystals, if specific substitute recommendations are superimposed.

The neutralization of formaldehyde is an unnecessary step, as commercial c.p. neutral formaldehyde (36% to 38%) can be easily obtained. Inferior grades of formaldehyde which are somewhat acidic, however, can be permanently neutralized by the addition of basic magnesium carbonate (Dunn and Loshakoff, 1936). Adjustment of the formaldehyde to pH 9.0 by means of sodium hydroxide is objectionable as a sediment is deposited on standing. A blank determination for the formaldehyde in the presence of the reagents used in the test compensates for the sodium hydroxide used to titrate the formaldehyde in the test. This is in disagreement with Pawlowski, who determines the blank on a water solution of formaldehyde.

TABLE I  
VALUE OF THE "BLANK" DETERMINATION IN THE FORMOL TITRATION

	0.1N NaOH, cc.					
	Formaldehyde and distilled H <sub>2</sub> O, pH 5.7		Formaldehyde and distilled CO <sub>2</sub> -free H <sub>2</sub> O, pH 7.0		Formaldehyde and reagents	
	North day- light	Day- light lamp	North day- light	Day- light lamp	North day- light	Day- light lamp
Thymol blue standard	1.0	1.0	1.0	1.0	0.7	0.7
Thymol blue and buffer, pH 9.0	1.0	1.0	1.0	1.0	0.7	0.7
Phenolphthalein and buffer, pH 9.0	1.5	1.5	1.5	1.5	1.3	1.3

### Choice of Indicators

Neutral red and phenolphthalein, the most commonly used indicators for the formol titration, require a color standard composed of a buffer solution with the indicator for comparison in measuring the initial and final pH of the test solution. Equally suitable indicators for which commercially prepared pH color standards are available are preferable to laboratory-prepared standards. Much time can be saved if the preparation of buffer solutions is eliminated.

Substitute indicators that may be considered are thymol blue for phenolphthalein and bromthymol blue or phenol red for neutral red. It is simply a question of the suitability of the substitute indicator.

Thymol blue of pH 8.0 to 9.6 has a sensitive range, *i.e.*, pronounced color change, of pH 8.4 to pH 9.2, with an optimum change at pH 8.8 (Britton, 1929). The end point (pH 9.0) is easily distinguished and the course of the titration is easy to follow. Likewise, bromthymol blue, pH 6.0 to pH 7.6, has a sensitive range of pH 6.4 to 7.2, optimum change at pH 7.1. Experiments involving the use of all the indicators mentioned disclosed that thymol blue is just as suitable as phenolphthalein, and that bromthymol blue is even preferable to neutral red. Results obtained in the presence of phenolphthalein in conjunction with the buffered color standard were the same as those obtained with thymol blue in conjunction with the buffer color standard as well as the pH (commercial) color standard (Table II). More consistent results were obtained with bromthymol blue than with neutral red; however, neutral red was at its  $pK_a$  at pH 6.8 and the end point was not so sharp. Phenol red, pH 6.8–8.4, may also be used in place of neutral red in fixing the initial pH of the test solution. Actually, with a little practice, the acid titration to pH 6.8 in the presence of phenol red may be accomplished without comparing the test solution with a color standard. The end point is reached at the complete permanent disappearance of the last trace of pink tint from the test solution.

TABLE II

VALUES OF AMINO ACIDS OBTAINED IN THE FORMOL TITRATION IN THE PRESENCE OF THYMOL BLUE AND PHENOLPHTHALEIN

Amino acid (pure solution)	Indicator used	Titrations, cc. of 0.1N NaOH			Mg. amino N	
		Total	Blank	Formol	Found	Present
Alanine	Thymol blue	6.0	1.0	5.0	7.0	7.0
Alanine	Phenolphthalein	6.5	1.5	5.0	7.0	7.0
Glycine	Thymol blue	6.0	1.0	5.0	7.0	7.0
Glycine	Phenolphthalein	6.5	1.5	5.0	7.0	7.0
IN THE PRESENCE OF ALL REAGENTS						
Alanine	Thymol blue	3.7	0.7	3.0	4.2	4.2
Alanine	Phenolphthalein	4.3	1.3	3.0	4.2	4.2
Glycine	Thymol blue	3.7	0.7	3.0	4.2	4.2
Glycine	Phenolphthalein	4.3	1.3	3.0	4.2	4.2

### Other Observations

The final concentration of formaldehyde by this method (9%) is the optimum for maximum accuracy (Levy, 1934).

Barium chloride need not be added in crystalline form, as the addition of a solution containing an equivalent amount of barium chloride gave the same results.

Carbon dioxide did not seriously affect the results unless the solution was unduly exposed, or delivery pipettes were blown through.

### Method

The procedure is modified as follows:

Pipette 60 cc. of extract or wort (12.5%) into a 100-cc. volumetric flask and immerse in boiling water for 10 minutes. Remove the flask, cool to room temperature, and treat with 10 cc. of 20% solution of barium chloride and 5 cc. of a saturated solution of barium hydroxide (7 cc. of barium hydroxide solution is necessary to provide an excess for proteolytic extracts), make up to 100 cc. with distilled water, shake and let stand for 30 minutes. Filter through No. 588 Schleicher and Schüll fluted filter paper or its equivalent (18.5 cm.), covering the funnel with a large watch glass.

Pipette four 20-cc. aliquots into four 125-cc. Erlenmeyer flasks (numbered 1, 2, 3, and 4), and plug with rubber stoppers. Prepare a blank solution in the same manner, substituting distilled water for the extract or wort.

To flask No. 1 add 2 cc. of 0.02% phenol red. To flask No. 2 add 2 cc. of distilled water. To Nos. 3 and 4 add 2 cc. each of 0.04% thymol blue.

Titrate flask No. 1 solution with 0.1 N hydrochloric acid to a distinct yellow (pH 6.8, the color should not immediately "fade" back). Add the same amount of hydrochloric acid required for flask No. 1 to each of flasks 2, 3, and 4. Add 10 cc. of 36% to 38% reagent-grade, neutral formaldehyde, pH 5.6 (formaldehyde of lower pH must be adjusted) to each of flasks 2, 3, and 4. Reject flask No. 1. Titrate the solution in No. 3 with 0.1 N sodium hydroxide to a near match with thymol blue pH 9.0 color standard (avoid overtitation—a little practice will aid in the judgment of approximate amount—or this titration may serve as an incremental titration in preparation for the titration of No. 4 solution). Add an equal amount of sodium hydroxide to flask No. 2. Compare solution 3 plus water ampoule with thymol blue color standard plus solution No. 2 in a color comparator, using daylight lamp or north light. A roulette comparator with a daylight lamp is most convenient for this purpose. If the test solution almost matches the standard, bring the solutions in flasks 2 and 3 to a total of 40 cc. each with distilled water and complete the titration to an exact match. A difference of 1 cc. in total volume in the test solution and solution No. 2 does not seriously affect the result. A more accu-



rate estimation is possible in the titration of solution No. 4. An analysis of the same solution on different days when the daylight varies in intensity will cause the results to vary. It is almost imperative that a standard daylight lamp be used for accuracy. The use of color standards of pH 9.0 on both sides of the test solution in the comparator permits a very close matching of colors. The addition of one drop of 0.1 N NaOH to a "matched" test solution should produce a color that, to the eye, is apparently darker than the standard.

The blank is determined in the same manner, except that ampoules of water are used in conjunction with the thymol blue standards instead of the compensating wort solution (flask 2).

The amino nitrogen is calculated as:

$$\% \text{ amino N}_2 = \frac{(T - B) \times 14 \times N}{M} \times 100,$$

where:

$T$  = cc. of NaOH (test solution titer)

$B$  = cc. of NaOH (blank solution titer)

$N$  = normality of NaOH

14 = mg. of nitrogen per cc. N NaOH

$M$  = mg. of dry matter (moisture excepted)  
represented in each aliquot.

Titration with  $0.107N \pm$  NaOH (0.106N to 0.108N) for 12.5% extracts, the equation becomes:

$$\% \text{ amino N}_2 = \frac{(T - B) \times 14 \times 0.107}{1500} \times 100 = (T - B) \times 0.1.$$

To convert to dry basis divide by  $(100\% - \% \text{ moisture of the malt})$ .

### Consistency of the Method

Walters (1937) obtained results by Kolbach's method varying from 0.1 cc. to 0.25 cc. of 0.1N sodium hydroxide, for duplicate estimations. It is assumed that the determinations were for duplicate aliquots of a given wort rather than determinations for duplicate extracts of a given malt.

The consistency of the method is best illustrated in Table III. A bulk sample of barley was thoroughly mixed and divided into two parts. Each part was subdivided into six equal parts. Two maltings were made under the same optimum conditions. Each sample of kilned malt was divided into two parts ( $a$  and  $b$ ) and separate extractions (worts) made. Each wort was analyzed in duplicate. The variance of the nitrogen in the wort is given as well as the amino nitrogen to show

TABLE III  
VARIABILITY OF RESULTS OF WORT NITROGEN AND AMINO NITROGEN

Sample number <sup>1</sup>		Wort nitrogen				Amino nitrogen in wort				Amino N/wort N ratio
		% Total D.M. wort	Mg. in 100 cc. wort	Mg. deviation from avg.	% Mg. N deviation	% Total D.M. amino	Mg. in 100 cc. wort	Mg. deviation from avg.	% Mg. N deviation	
1	1a	.560	70.0	-0.3	-0.42	.185	23.6	+0.2	+0.85	33.03
	1b	.559	69.9	-0.4	-0.57	.175	22.3	-1.1	-4.70	31.30
2	2a	.566	70.8	+0.5	+0.71	.190	24.2	+0.8	+3.41	33.60
	2b	.562	70.3	0.0	0.00	.190	24.2	+0.8	+3.41	33.80
3	3a	.568	71.0	+0.7	+1.00	.180	23.0	-0.4	-1.71	31.70
	3b	.567	70.9	+0.6	+0.85	.185	23.0	-0.4	-1.71	31.74
4	4a	.570	71.3	+1.0	+1.42	.185	23.6	+0.2	+0.85	32.45
	4b	.560	70.0	-0.3	-0.42	.185	23.6	+0.2	+0.85	33.03
5	5a	.564	70.5	+0.2	+0.28	.185	23.6	+0.2	+0.85	32.80
	5b	.556	69.5	-0.8	-1.14	.185	23.6	+0.2	+0.85	33.27
6	6a	.556	69.4	-0.9	-1.28	.180	23.0	-0.4	-1.71	32.43
	6b	.563	70.4	+0.1	+0.14	.185	23.6	+0.2	+0.85	32.85
Average range		.563 .015	70.3 1.9	— 1.9	— 2.70	.184 .015	23.4 1.9	— 1.9	— 8.11	32.66 2.5
7	7a	.569	71.1	+0.2	+0.28	.195	24.9	+0.2	+0.81	34.27
	7b	.564	70.5	-0.4	-0.56	.195	24.9	+0.2	+0.81	34.57
8	8a	.570	71.3	+0.4	+0.56	.195	24.9	+0.2	+0.81	34.21
	8b	.570	71.3	+0.4	+0.56	.195	24.9	+0.2	+0.81	34.21
9	9a	.564	70.5	-0.4	-0.56	.195	24.9	+0.2	+0.81	34.57
	9b	.568	71.0	+0.1	+0.14	.195	24.9	+0.2	+0.81	34.33
10	10a	.565	70.6	-0.3	-0.42	.195	24.9	+0.2	+0.81	34.51
	10b	.579	72.4	+1.5	+2.10	.201	25.6	+0.9	+3.64	34.71
11	11a	.565	70.6	-0.3	-0.42	.195	24.9	+0.2	+0.81	34.51
	11b	.561	70.1	-0.8	-1.12	.190	24.2	-0.5	-2.02	33.84
12	12a	.559	69.9	-1.0	-1.40	.185	23.6	-1.1	-4.45	33.09
	12b	.569	71.1	+0.2	+0.28	.185	23.6	-1.1	-4.45	32.51
Average range		.567 .020	70.9 2.5	— 2.5	— 3.50	.193 .016	24.7 2.0	— 2.0	— 8.09	34.11 2.2
Grand average		.565	70.6	—	—	.189	24.1	—	—	33.4
Grand range <sup>2</sup>		.024	3.0	3.0	4.30	.026 <sup>3</sup>	3.3	3.3	13.60	3.4

<sup>1</sup> Each value given is the average of duplicate determinations. Extractions *a* and *b* are duplicate. Samples 1 to 6 and samples 7 to 12 are replicates. Samples 1 to 6 and 7 to 12 are duplicate maltings.

<sup>2</sup> Represents 0.6 cc. of 0.1N NaOH (wort N).

<sup>3</sup> Represents 0.26 cc. of 0.107N NaOH (amino N) (over all titration range).

the variability within the sample, in malting, and within the wort, as determined by analysis of the nitrogen in the wort.

An example of variance between extracts is best illustrated by sample 10*b* (Table III). Although the mg. deviation from the mean deviation for amino nitrogen is considerable, the mg. deviation from the mean for the corresponding wort nitrogen is equally noticeable.

The values given in Table III are the averages of duplicate determinations. Of 24 duplicate determinations made, 22 checked exactly, and 2 within 0.6375 mg. of nitrogen in terms of 100 cc. of wort. Eight duplicate worts checked exactly and 4 checked within 0.6, 0.7, 1.3, and 1.4 mg., respectively, per 100 cc. of wort. Apparently, variation in values obtained may be attributed considerably to variable malting conditions and variability within the sample and within the extract.

### Summary

Practically pure neutral formaldehyde is easily obtained and should be used in preference to cloudy formaldehyde that sediments on standing. Inferior grades are objectionable as they interfere with the continuity of procedure and the technique of the analyst.

The blank for the formaldehyde is useless unless determined in the presence of the reagents used in the test.

The use of a standard commercial set of pH color standards is more convenient than the use of buffer solutions used in conjunction with indicators. The pH of the buffer solutions is subject to change from day to day due to handling and exposure to carbon dioxide in the atmosphere. The preparation of the buffer solutions is time consuming and tedious.

The use of phenol red and thymol blue indicators is preferable to neutral red and phenolphthalein indicators, respectively. The amount of indicator in the test solution must be equal to the amount in the color standard.

Greater accuracy is possible by use of a daylight lamp and comparator combination, than by daylight.

The modified method, simplifying Pawlowski's method, is convenient, consistent, and adaptable to routine purposes.

### Literature Cited

- American Association of Cereal Chemists  
1935 Cereal Laboratory Methods.  
Association of Official Agricultural Chemists  
1935 Methods of Analysis.  
Britton, H. T. S.  
1929 Hydrogen Ions. Chapman and Hall, Ltd., London.  
Dunn, M. S., and Loshakoff, A.  
1936 Amino acids and peptides, I. J. Biol. Chem. **113**: 359-369.  
Folin, O.  
1922 A system of blood analysis. J. Biol. Chem. **51**: 377-391.  
Foreman, F. W.  
1920 Rapid volumetric methods for the estimation of amino acids. Biochem. J. **14**: 451-473.  
Kolbach, P.  
1928 Über die Ausföhrung der formol titration. Wochschr. Brau. **45**: 446-451.  
Levy, M.  
1934 Acidity of formaldehyde. J. Biol. Chem. **105**: 157-165.

- Pawlowski, F.  
1932 Die Brautechnischen Untersuchungs-methoden. München und Berlin. Verlag von R. Oldenbourg.
- Richardson, G. M.  
1934 Formaldehyde, alcohol and acetone titrations. Proc. Roy. Soc. London **115**: 121-141.  
Biological estimation of amino nitrogen. Proc. Roy. Soc. London **115**: 142-169.
- Schryver, S. B., and Thomas, E. M.  
1929 The nitrogenous constituents of wort. J. Inst. Brewing **35**: 571-576.
- Sørensen, S. P. L.  
1907 Etudes Enzymatiques. Compt. rend. trav. lab. Carlsberg **7**: 1-57.  
1907 Enzymstudien. Biochem. Z. **7**: 45-101.  
1909 Ergänzung zu der Abhandlung: Enzymstudien II: Über die Messung und die Bedeutung der Wasserstoffionenkonzentration bei Enzymatischen Prozessen. Biochem. Z. **22**: 352-356.
- and Hansen, H. J.  
1908 Sur la titration au formol de solutions fortement colorées. Compt. rend. trav. lab. Carlsberg **8**: 58-136.
- Van Slyke, D. D.  
1911 A method for quantitative determination of aliphatic amino groups. J. Biol. Chem. **9**: 185-204.  
1912 The quantitative determination of aliphatic amino groups, II. J. Biol. Chem. **12**: 275-284.  
1913 The gasometric determination of aliphatic amino nitrogen in minute quantities. J. Biol. Chem. **16**: 121-134.  
1929 Manometric determination of primary amino nitrogen and its application to blood analysis. J. Biol. Chem. **83**: 425.
- Walters, L. S.  
1927 The estimation of amino-acid nitrogen in brewing materials with especial reference to Folin's colorimetric method. J. Inst. Brewing **34** (n.s.): 44-51.

## REPORT OF THE 1938-39 COMMITTEE ON METHODS OF ANALYSIS

R. M. SANDSTEDT, *Chairman*

Department of Agricultural Chemistry, University of Nebraska,  
Lincoln, Nebraska

(Read at the Annual Meeting, May 1939)

The members of the Methods Committee during the past year have conducted the following investigations of methods: the gas retention of doughs, the evaluation of yeasts for baking purposes, a collaborative study of the 15-minute moisture method, and a study of methods of determining proteolytic activity. The study of methods of titration of mono-calcium phosphate was postponed because of the introduction of a new type of mono-calcium phosphate which might necessitate some adjustments in the methods.

### Recommendations

1. That the study of methods for evaluating yeasts be continued.
2. That a study be made of methods for the titration of mono-calcium phosphate.

3. That the investigation of gas retention be continued.
4. That a collaborative study be made of the Binnington-Geddes rapid method for determining wheat and flour pigments.
5. That the collaborative study of the 15-minute moisture method be completed.
6. That a collaborative study be made of experimental milling.
7. That the study of methods of determining proteolytic activity be continued.

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### BOOK REVIEW

**Getreidelagerung, unter besonderer Berücksichtigung der bauerlichen und landwirtschaftlichen Verhältnisse.** By Kurt Seidel, B. Czyżewsky, and W. Hammer. Second edition. Schriften des Reichskuratorium für Technik in der Landwirtschaft, Heft 58. Beuth-Vertrieb GmbH, Berlin, 1938. 150 pages, illustrated. Price RM 4 (paper covers).

This little manual is designed primarily to instruct the farmer in the best methods of handling newly harvested grain in the field and in the granary. Aside from this, however, it presents much valuable scientific information on the storage of grain in large quantities, and includes chapters on artificial drying of grain and storage of flour and feed.

A good share of the information in this book has to do with the precautions necessary to prevent respiration losses and damage in moist grain. Because the harvest season in Germany is frequently accompanied by rains and high humidities, the average moisture content of the grain when harvested rarely runs below 15%–16% in normal years, while in wet years it mounts to 18% or higher. Only in very dry years does the moisture content fall below 15%. Some years ago Hoffman estimated that the losses due to unfavorable harvest weather amounted to 60 million marks in dry years and 250 million marks in wet years. Other authors have estimated that in wet years the losses may run as high as 10% of the total crop. Aside from these losses, the value of the grain is impaired by sprout damage, and the high moisture content necessitates either artificial drying or frequent moving during storage.

The book opens with a brief chapter by K. Seidel, outlining in non-technical language "what every farmer must know" about the storage of his grain, and indicating the precautions necessary to prevent excessive respiration losses and insect damage.

This simple outline is elaborated upon in the next section, by B. Czyżewsky, which comprises the bulk of the book. This is an able scientific discussion of many of the factors involved in the storage of grain on the farm and in large commercial granaries and elevators. Respiration, sweating, adsorption of moisture and odors, and infection with microorganisms are touched upon. Precautions to be observed during storage of unthreshed grain in stacks and in sheds are mentioned.

In connection with the discussion of sprout damage, attention is called to the work being done at the Leipzig Institut für Pflanzenbau, in selecting grain varieties which sprout less readily at the high moisture levels common in German grain, especially wheat and rye. It is anticipated that the widespread cultivation of these varieties will considerably reduce losses due to sprout damage.

Methods of combating insect pests of stored grain are set forth at some length, and information is given on the use and efficacy of a wide variety of insecticides.

Various methods of effecting natural or forced ventilation of granary floors are described and illustrated, as are also ventilating systems for large and small grain elevators. A useful grain aeration table is included, from which can be determined the maximum relative humidity permissible in the air admitted for aeration, in order that it shall not carry more than 75% relative humidity when it has attained the temperature of the grain.

The storage of flour and bran is the subject of a short chapter by K. Seidel. It points out that in order to prevent further increase in moisture content and possible spoiling, aeration of warehouses containing moist flour should not be undertaken unless the relative humidity of the outside air is less than 75% and its temperature not more than 3°C. lower than that of the flour. Effects of damage by fresh and salt water on sacked flour are discussed.

The principles of artificial drying of grain are outlined by W. Hammer, and 10 types of German grain dryers are described.

A supplement presents 70 questions and answers dealing with storage of grain and cereal products. This serves as a summary of the foregoing chapters, and includes information not previously given, especially with regard to the milling quality of damaged grain.

CLINTON L. BROOKE

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**Cereal Chemistry, Agricultural Experiment Station, Lincoln, Nebraska.**

## THE PRODUCTION OF MECHANICALLY DAMAGED STARCH IN MILLING AS A GOVERNING FACTOR IN THE DIASTATIC ACTIVITY OF FLOUR

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(Received for publication July 21, 1939)

During the last year or two notable contributions to the literature have appeared<sup>1</sup> giving attention to the nature and condition of the starch as the origin of differences in diastatic activity of flours. Sandstedt, Blish, Mecham, and Bode (1937) deduced from the observed course of diastatic activity with time that the higher activity found with commercial (American) flours than with laboratory-milled was due to the fact that the former contained larger proportions of the more susceptible starch fractions, in other words greater amounts of ruptured starch granules. Blish, Sandstedt, and Kneen (1938) point out that autolytic diastasis in flour is very rapid at first but after an hour or more diminishes to a very low rate, a fact attributable to exhaustion of the available starch substrate, not to exhaustion of active enzyme. They further point out that the small amount of the starch normally converted is substantially increased through additional grinding of the flour as in a ball mill and that it is apparent from their data on autolysis that a portion of the "susceptible" starch (susceptible to beta-amylase attack) in flour is a result of the mechanical rupture of raw starch during milling. They add that it seems likely, however, that at least a significant amount occurs naturally in the form of dextrans or closely related substance.

L. H. Pulkki (1938) observed marked increases in maltose figure and gassing power in samples of middlings ground to pass increasingly fine silks. He attributed this to the increase in the proportion of mechanically damaged starch granules which could be stained with congo red.<sup>2</sup>

<sup>1</sup> Since the preparation of this paper for publication, O. E. Stamberg and C. H. Bailey (1939) have reported that although alpha-amylase (made from germinated wheat) can hydrolyse substantial proportions of raw wheat starch, beta-amylase (made from normal wheat) is able to hydrolyse only small amounts. They show however that when the same wheat starch had been severely mechanically damaged it was hydrolysed as easily as soluble starch paste by both alpha- and beta-amylase.

<sup>2</sup> Actually C. L. Alsberg and E. P. Griffing (1925) had covered substantially the same ground in this respect except that they dealt with the amount of cold water extract obtainable instead of maltose figure. They observed (qualitatively) that the proportion of starch granules stainable with congo red increased markedly as a sample of flour was ground to pass progressively finer silks. They in turn were directed to the use of congo red solution by an earlier finding of H. Huss (1922).



He considers that the starch granules of wheat have a separate thin protective surrounding layer or film, which unlike the inside material of the granule is not easily attacked by diastase nor stainable with congo red but can easily be removed by mechanical means. This in the present writer's view comes very near to the truth but does not quite accord in a few important particulars with some observations to be described. This and certain other aspects of Pulkki's paper will be further considered in appropriate places in the present paper. It might however be added here that Pulkki thinks that "if some entirely different method of grinding (other than rolls) were used, it would be at least theoretically possible that a high degree of fineness could be reached without affecting very great changes in amylolytic activity of the material."

The conclusions and views cited above had in fact formed a material part of a position reached by the writer some years ago as set out in Reports of the Research Association of British Flour-Millers<sup>3</sup> and he is naturally gratified by the support now appearing for his observations and deductions. In view of the extent of the interest now taken in this field he feels it may be of interest and service to other workers to put forward some of the further conclusions arrived at during the work in question.

It might be illuminating to describe briefly how the matter came to receive attention in the first place. A laboratory mill had been in use in the St. Albans laboratories for many years and in 1933 a second new mill was obtained and put into additional operation. Comparisons of flours made from similar wheat samples on the two mills, after careful attention to equivalent operation in every obvious particular, showed marked differences in maltose figure and gassing power. Systematic tracing of the factor responsible made it clear that the difference in reduction-roll surface was responsible. The rolls of the new mill had a smooth, polished surface; the old were rather rough and pitted.

### **Microscopical Appearance of Starch Granules Damaged during Milling**

The microscope was then used to ascertain the nature of the change in the flour leading to raised maltose figure as a result of the use of rather rough reduction-roll surfaces as against smooth under quite similar conditions. In the first instance it was not known what to expect beyond the probability that one flour would show some form of starch damage absent from the other. One expected (*a priori*) to

<sup>3</sup> Confidential Reports of the Research Association of British Flour-Millers, Nos. 28, 30, and 31, issued in February, September, and October, 1934.

see some of the granules cracked or split, probably radially on the lines of the preparation shown in Plate 1.<sup>4</sup>

This type of damage, however, does not occur in flours freshly roller-milled, and it may be said at once that the type of damage which actually is found associated with an increase in maltose figure, due to one form of rolling as opposed to another, is less obvious, though it is necessarily seen to varying extents whenever any flour is examined microscopically.

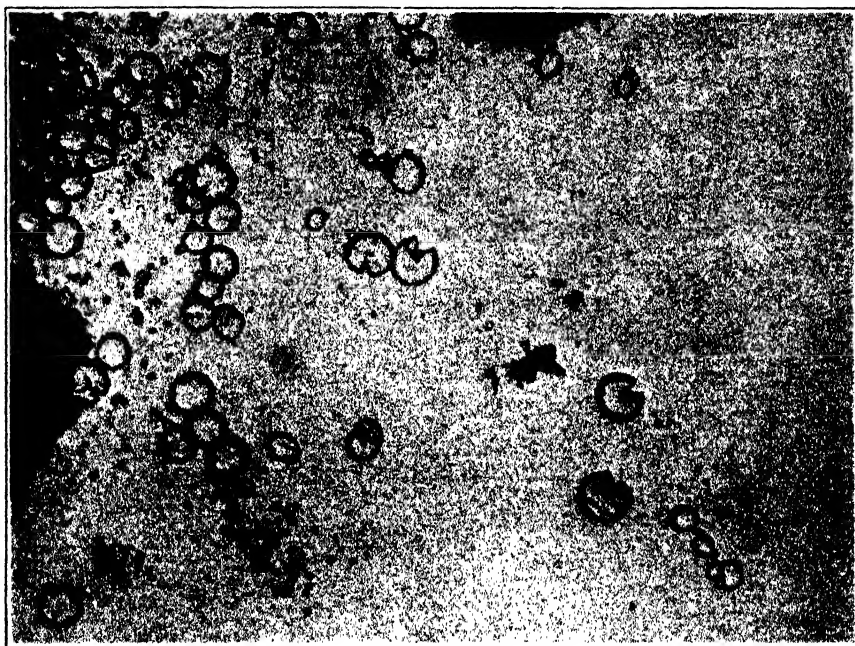


PLATE 1

Granules of wheat starch cracked radially through hand-manipulation on the slide.

(All magnifications are approximately  $\times 120$  diameters)

The damage is nevertheless highly characteristic. A flour which has been fairly severely milled (using the word "severely" in the sense of conditions which make for production of high maltose figure) is shown in Plate 2A.

The starch granules in question which have sustained damage are indicated by a star on the photograph. They do not look cracked but have a curious flat appearance and thin faint outline. Compare for example *a* in Plate 2A, with the sound granules marked *b* or *c* (the latter in the upper left-hand corner). Their attenuated appearance in contrast to the boldness of sound granules suggested the term "ghosts,"

<sup>4</sup> The magnification in each photograph is approximately 120 diameters.

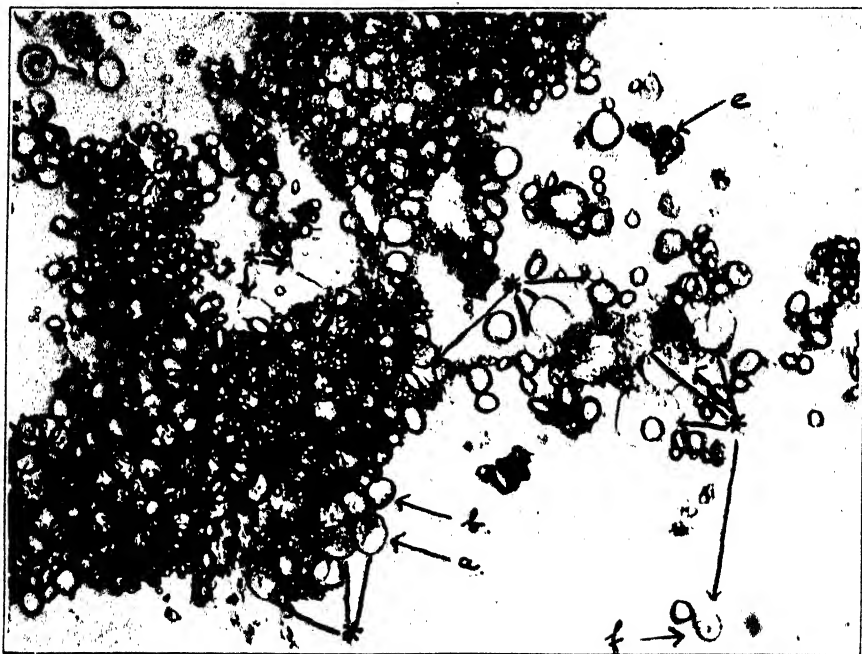


PLATE 2A

Wheat flour showing "ghosts."

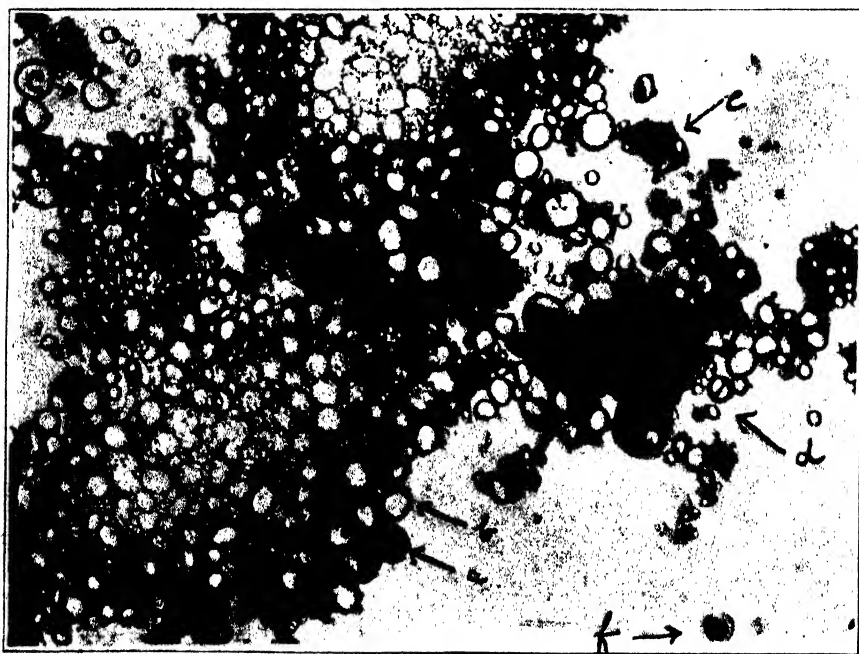


PLATE 2B

Same preparation as 2A afterwards stained with congo red.

which was afterwards found not inconvenient in referring to this particular type of starch damage.

"Ghosts" are more easily detected in flour through suitable differential staining, a phenomenon which is also of significance in regard to the part they play in diastasis. Dilute aqueous congo red solution is excellent for the purpose (0.1% to about 0.35% is a suitable concentration). It does not stain sound starch whilst the "ghosts" all stain uniformly an orange-pink. In flour preparations it stains gluten a rather vivid brown. Care is sometimes required (especially with low-grade flours) to avoid confusion with pieces of endosperm cell-wall tissue, which stain a vivid pink.

Plate 2B shows the same preparation as 2A after staining <sup>6</sup> through irrigation with 0.35% congo red solution. It is interesting to compare now the appearances of the granules referred to above and also to note how the colony of "ghosts" at *d* in Plate 2B is brought into prominence. Incidentally the gluten, which, in the form of its delicate meshwork permeating the large clumps of starch granules, is only indistinctly apparent in Plate 2A, is dyed intensely by the stain and its extent becomes sharply defined in the photo. This is well shown, for example, by the very small endosperm particle *e* in Plates 2A and B, and also in patches at *d*. With the very large particles, however, the stain is taken up eagerly by the gluten of the outer margin, the interior escaping staining as is well seen in Plate 2B.

The results of staining by irrigation are easily deceptive in many ways in that uniform distribution of the stain over the field cannot be brought about. In Plate 2B, for example, the stain streamed in from the lower edge of the photo with the consequence that the upper edges of particles tended rather to escape staining relative to the lower. The streaming also produces a certain mechanical derangement of the objects under view. The relatively large mass of which *a* and *b* are outlying granules has thus been shifted upwards, approaching the upper mass of roughly equal size. The gulf or inner sea initially separating the two has thus been contracted and partly forced out towards *e*. The currents of plain water so formed further upset the distribution of the dyestuff. The upshot is that many of the particles seem from the photo to show an irregularity or non-uniformity of staining which is apparent rather than real. In the great bulk of the cases to be described, uniformity of staining was obtained by making up the preparation at the start with the stain solution.

A few very important features of this staining must now be mentioned. It is uniform: starch granules either stain alike or not at all.

<sup>6</sup> The effect is difficult to register photographically with the vividness presented to the eye. The artifice was found helpful of using green light in conjunction with ortho-chromatic plates.

Every stained granule has the same tint, and each granule is uniformly tinted through its stained part. It is possible very occasionally to see a granule which is partly sound and partly "ghost," shaped thus:



the part *a* having the "solid," strongly outlined appearance of sound starch and *b* the customary faint, enlarged outline of the "ghost." In such a case *b* stains uniformly, *a* remains unstained. Apart from such rare cases the "ghosts" are invaded by the stain from all around the circumference equally. This may be seen if, instead of making up the slide initially with dye solution, an attenuated preparation with water only is made and irrigated with the congo red solution under the microscope. The zone of stained material in any given "ghost" increases in width uniformly from the periphery inwards as the colour encroaches evenly until the last unstained point at the center has disappeared leaving the "ghost" uniformly tinted. This process is exceedingly rapid with congo red solution; it happens so quickly that it is not easily followed. It is slower and much more easily followed with sufficiently dilute iodine solution.

Iodine (in an aqueous solution of potassium iodide) of more than a certain concentration stains all starch granules, sound or damaged, indiscriminately a very deep purplish blue. Such an effect is shown in Plate 3A, the solution used containing 0.3% iodine. On irrigating a flour-water slide with such an iodine solution, however, it is possible to see the "ghosts" take up the colour much more quickly, and at first more deeply, than the sound granules. It was then found that if the iodine solution used for making up a slide is sufficiently dilute the "ghosts" will stain intensely blue, whereas the sound granules will never stain more than a very faint mauve. A solution containing 0.02–0.03 g. iodine and 0.07 g. potassium iodide in 100 g. of water gives in this way an exceedingly sharp differentiation between "ghosts" and normal granules. The effect is shown on Plate 3B. Apart from intensity, the colours of the normal granules and the "ghosts" are distinct, the former being purple and the latter pure blue.

A further important feature of the staining phenomena is that the process (whether with iodine or with congo red) can be reversed. If the stained slide is irrigated with pure water the stain disappears from the "ghosts," from the outer margins evenly first and lastly from the center. The process can be repeated many times, fresh dye being introduced and then removed. After several repetitions, however, the intensity of the stain taken up by the "ghosts" becomes much less.

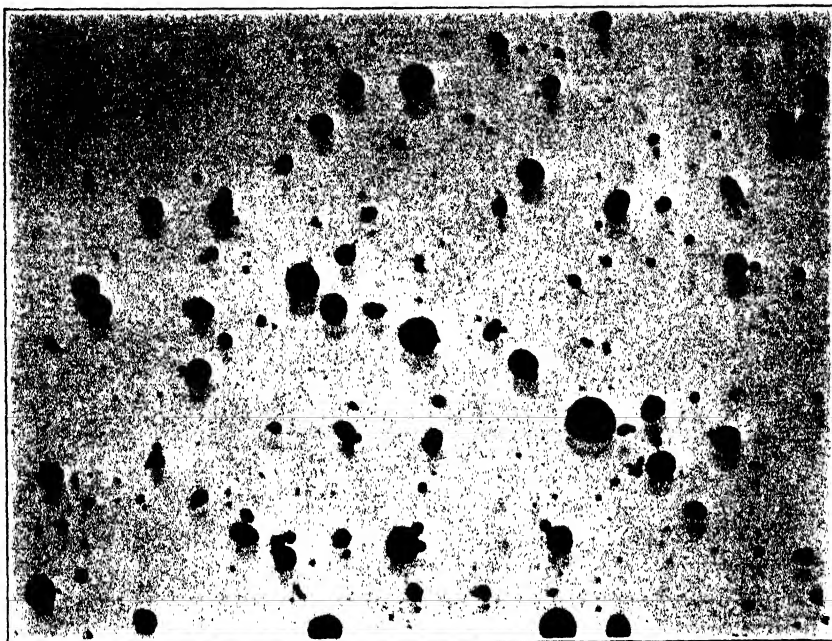


PLATE 3A

Wheat starch, partly sound and partly "ghosts," stained with 0.3% iodine solution.

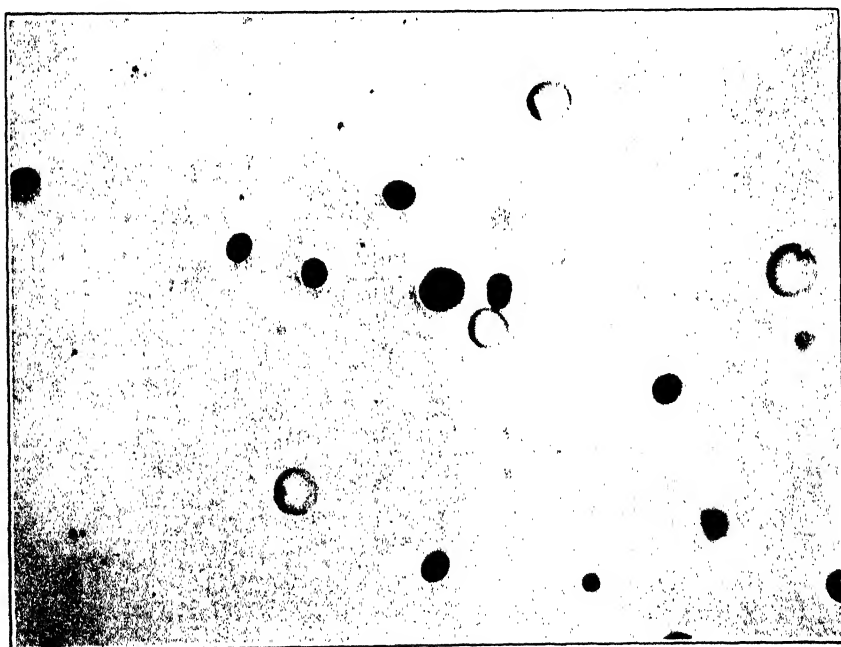


PLATE 3B

Wheat starch, partly sound and partly "ghosts," also with a radially cracked granule, stained with 0.02% iodine solution.

### Some Different Forms of Mechanical Damage to Wheat Starch Granules

The larger granules of wheat starch may be regarded as playing the predominant part in the characteristic phenomenon under discussion. Usually reported as of 30 to 35  $\mu$  in diameter, they are lenticular in shape but ordinarily are seen lying flat, with a circular outline. Mechanically they are very delicate structures and if gentle pressure is applied to the cover slip whilst the granules are examined under the microscope it may be seen that they are very easily flattened with, of course, a resulting increase in the diameter of their circular outline. They have marked elastic properties. If with a microscope slide of a mixture of flour or starch and water the cover slip is gently pressed, any given granule affected by the pressure expands perfectly uniformly in area as viewed from above, and on release of the pressure returns immediately to its original shape. If now the cover slip is pressed rather more firmly in a "springy" fashion and released, pressed again and released, the process being repeated several times (preferably, it is thought, with a slight interval between the releasing and re-pressing) the granules crack or split in a characteristic radial fashion, the damage produced being shown in Plate 1.

If, however, a fresh slide is made and the cover slip handled rather differently, an entirely different type of damage is produced. In this process a much heavier pressure is applied gradually and maintained for a little time so that the granules are not released but kept just beyond what might be called the limit of their elastic deformation. On now releasing the pressure the granules do not recover as in the first case. They are permanently flattened and have become in fact "ghosts." As a very rough estimate they now have 50% greater diameter than at first.

If the treatment is rough the "ghosts" produced are extensively cracked or fissured finely in a radial fashion for some little distance inwards from the periphery; with very rough treatment they can eventually be broken up. In fact, it requires some practice to produce the ghosts "artificially" in this way in the perfect condition in which they are nearly always found in flour as a result of ordinary milling processes. Perfection of condition is here spoken of as meaning freedom from all cracks or fissures and easily visible flaws, the "ghosts" as mentioned previously having characteristically the same outline appearance viewed from above as the sound granules. Plate 4A shows "ghosts" made by hand manipulation in the way described, the granules in question having all been sound when the slide was first made. The most nearly perfect examples are denoted by an X marked on the photograph.

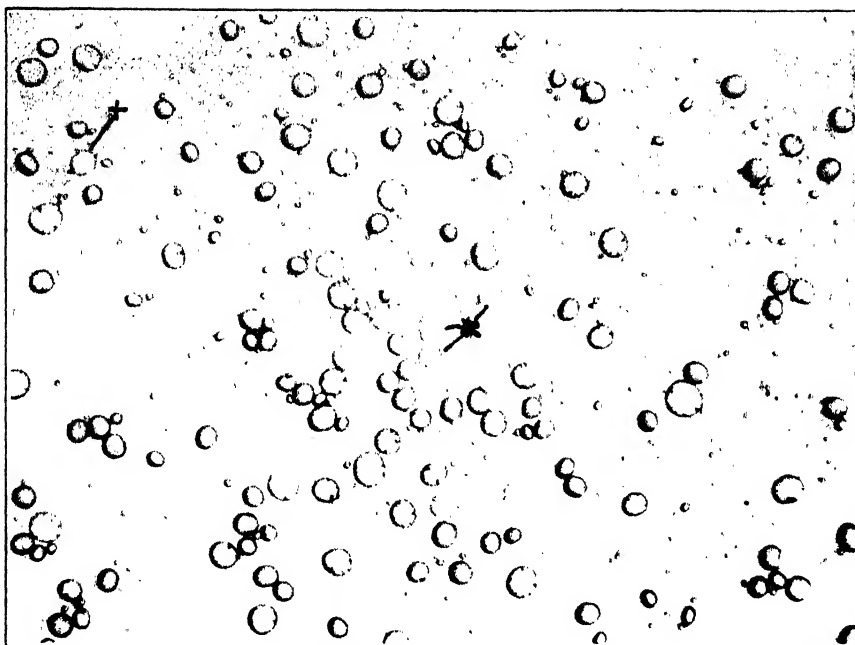


PLATE 4A

Wheat starch containing some "ghosts" made by hand-manipulation on the slide, unstained.

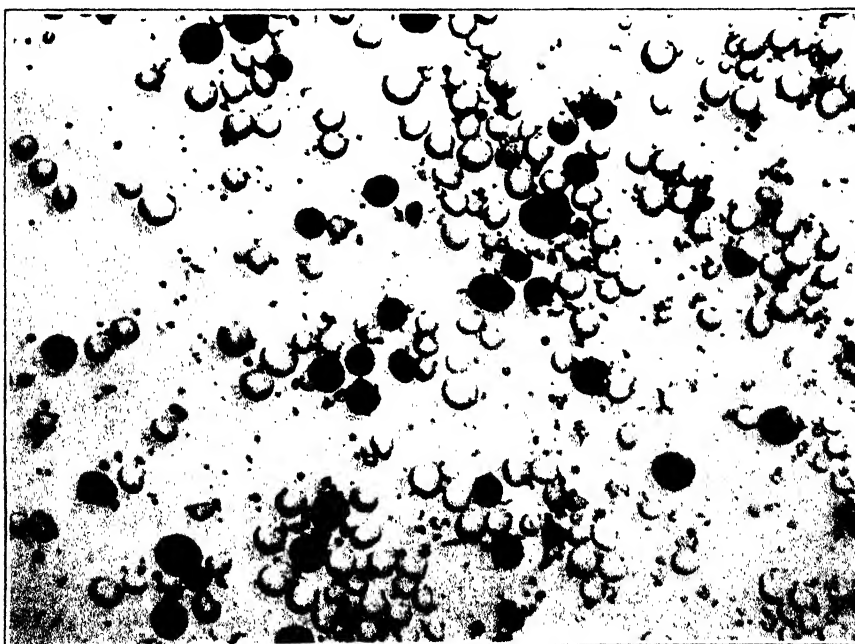


PLATE 4B

Wheat starch containing some "ghosts" made by hand-manipulation on the slide, stained with 0.03% iodine solution.



In every way, as far as can be observed under the microscope, the "ghosts" so produced behave the same as those found in the first place in flours. They stain in exactly the same way with congo red or the 0.03% iodine solution. Plate 4B shows a preparation in which some of the granules have been made into "ghosts" by hand manipulation: all such are stained with 0.03% iodine. On the other hand, the radially cracked "solid" granules do not stain except perhaps for a very slight margin on the edges of the larger fissures when these are V-shaped: they behave like the sound granules with respect to staining reagents. Plate 4C gives a very clear view of a few granules in 0.02% iodine solution. One is a "ghost" made by hand manipulation and has stained at once, in contrast to the others, two of which are sound and two radially cracked.

It is thought, however, that on long standing the radially cracked granules slowly take up the stain, eventually becoming uniformly

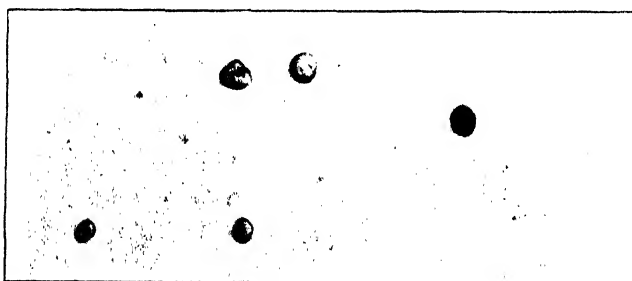


PLATE 4C

A small group of wheat starch granules in 0.02% iodine solution.

stained throughout, whereas the perfectly sound granules remain unaffected in the presence of the stain.

We thus have two characteristic, different forms of damage to which the starch granule may be subject of which one, the "ghost" type, is specifically a result of flour-milling processes generally.

It should be emphasized that the fissuring of the starch granules produced during *germination* of the grain is a quite distinct type of damage. As is well known the granules are "pitted" under these conditions. Even at an advanced stage, however, when badly eroded starch is concerned, the granules so attacked do not stain with congo red,<sup>6</sup> though it was found that the 0.03% iodine solution will stain them, not however a pure blue as in the case of the "ghosts," but a deep purplish colour.<sup>7</sup>

<sup>6</sup> This phenomenon is one of the pointers indicating that in ordinary damage of wheat starch it cannot be a question of peeling off a protective skin as suggested by Palkki.

<sup>7</sup> It is outside the scope of this paper to discuss non-mechanical means of damaging starch granules, *vis.* by heat in presence of water or by means of chemical reagents. It may however be pointed out that granules heated in water to just above the gelatinisation temperature appear, stain, and behave like "ghosts" produced mechanically as described above.

### One Type of Damage in Milling Practice

The point the writer wishes to make now is that of these various types of damage it is only the "ghost" type that is produced during ordinary flour-milling operations. It will presently be shown that a number of very distinct mechanical factors in flour-milling reduction processes produce damaged starch, but always the granules affected are in the form of "ghosts." Moreover they are produced exceedingly easily and it is not surprising that all commercial breadmaking flours contain appreciable though varying amounts. In fact, unless the grain is well soaked to soften it, it is impossible to cut a wheat grain and remove particles of endosperm from the cut surface with a knife, however gently, without damaging much of the starch. Again the striking thing is that starch so damaged is in the form of "ghosts." By shearing (as in scraping) or by heavy pressure (as on the microscope slide) the one characteristic type of damage is produced—a point which is of practical importance. Another point no less important is a property of the "ghosts" when found under certain circumstances of occurring in *aggregates* which are always in the form of flattened flakes.

A small flake or sheet of "ghosts" may be seen for example attached to the "southwest" side of the flour particle near the center of Plates 5A and 5B. Often the flakes are much more extensive than this and they may be found in particles much larger than those of flour, *e.g.* dunst.

On gently rubbing the cover slip over the slide the colonies of "ghosts," whether alone or attached to a starch-gluten aggregate, are clearly seen to have relatively considerable cohesion. They hang together very well whereas the sound starch, once it is gently freed from the gluten meshwork, comes teeming out in discrete granules. It will be shown later how this property is of practical importance.

### The Relation between Number of Ghosts and Diastatic Activity

Plates 5A and 5B show a picture of a low-grade flour (*L-roll flour*) which had a maltose figure of 3.5%, having come from a grist rather abnormally rich in diastase. As distinct from higher-grade flours, no very large aggregates are to be seen, the starch granules being more or less scattered, and amongst the larger granules the "ghosts" outnumber the sound granules.

On standing overnight a remarkable change was seen in the slide of the *L-roll flour*. Hardly any "ghosts" could be seen, the starch being present as sound granules, which did not appear in any way altered. Fresh irrigation with congo red solution produced no further staining at any point in the slide. It is not possible to show the

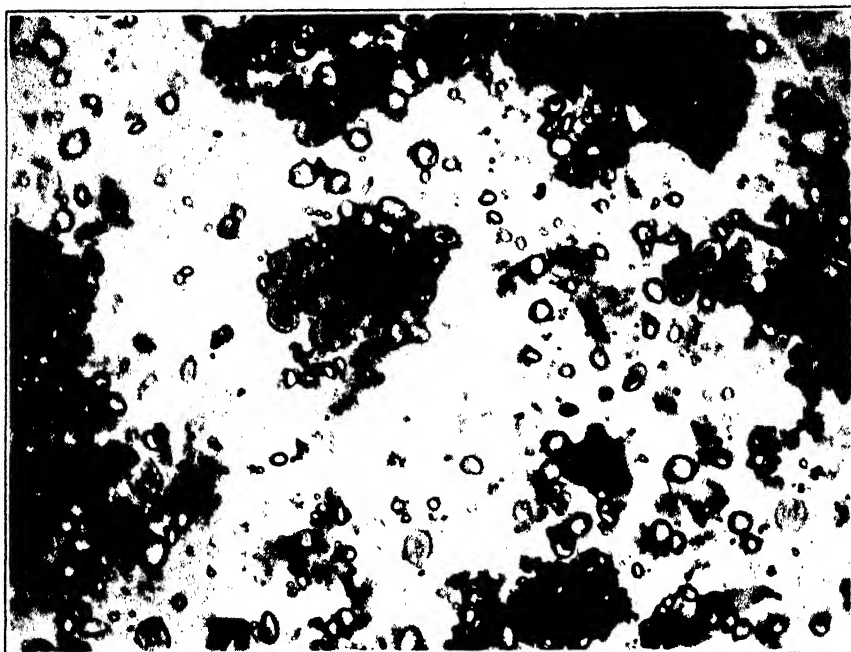


PLATE 5A

An *L*-roll flour (maltose figure 3.53%) (stained with congo red).

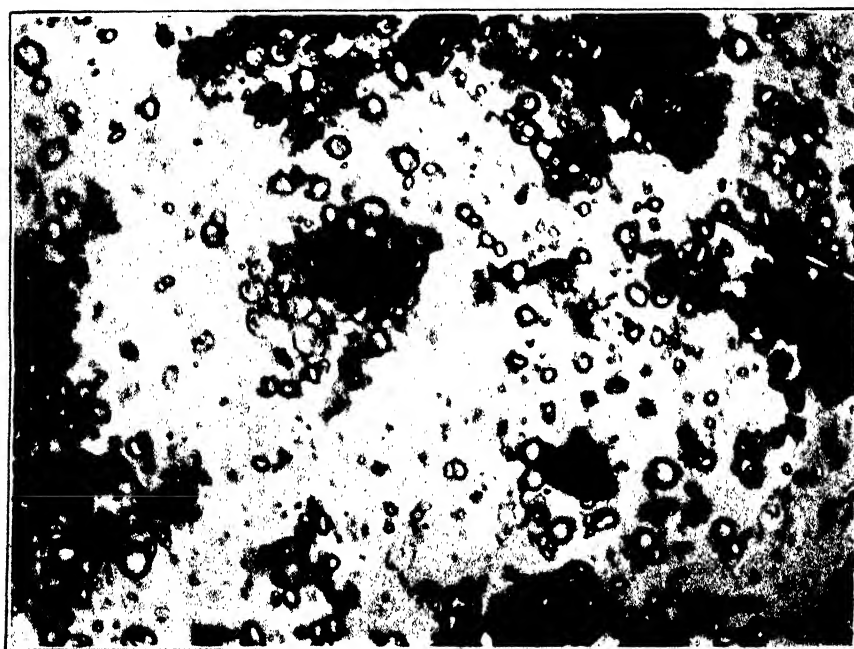


PLATE 5B

The same preparation as 5A after standing untouched a few hours.

phenomenon here in the marked degree described but nevertheless comparison of Plate 5B with 5A is very informative. Plate 5B was the same subject taken after standing a few hours, 5A, of course, having been taken immediately the slide was made up. It will be seen, for example, how the big patch of "ghosts" near the center has almost faded away, whereas the initially sound granules are in general unaffected. By contrast a stained slide of laboratory-milled low-grade flour from Plate wheat (of low diastatic activity) which had maltose figure 1.0%, showed relative permanence in what "ghosts" it contained; even after standing two or three days the "ghosts" were visible, distinctly stained. If such a flour, or even commercial wheat starch, is made up on the slide with a solution containing highly diastatic malt extract in addition to the congo red stain, any "ghosts" that are present practically disappear in less than ten minutes at room temperature, the sound granules appearing unaffected.


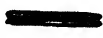
*In general with flours differently milled from the same wheat the order of abundance of "ghosts" is in the same direction as the maltose figures.* Moreover, the range of maltose figure which can be covered in the case of samples of flour milled from a given sample of conditioned wheat is very great. Thus in the case of No. 2 Manitoba wheat, flour of maltose figure 1.0% was obtained with a very gradual laboratory milling system (gentle reductions, low releases, and correspondingly numerous passages through the rolls) in which highly smooth reduction rolls were used. By the use of a similar system with rather rough, pitted reduction rolls on the same sample of conditioned wheat, a similar length of flour of maltose figure 1.9% was made. Using the rougher rolls with fairly heavy pressures and fairly high releases and correspondingly fewer reductions, the maltose figure was 2.2%. Moreover, if any of the finished flours were taken and put a few consecutive times through closely engaged smooth rolls the maltose figure could without difficulty be raised to something of the order of 5%, an observation in line with those of several other workers.

### **Damaged Granules in Diastasis**

The facts so far given led, as has since been substantially recognized by other workers, to the position that only the "ghosts" could be regarded as undergoing diastatic conversion in ordinary flour doughs or suspensions.

It is outside the scope of this paper to consider closely the structure of the starch granule and the various conceptions advanced by many workers. It will suffice to make the simple statement that the soluble "inner starch" is ordinarily protected by an envelope or skin of

insoluble "outer starch" surrounding the granule. The former alone is fermentable by the diastatic agent present in ordinary sound non-germinated wheat flour. This agent cannot act until the outer resistant skin of the granule is broken, either by mechanical means or through heat gelatinisation. Therefore the "ghosts" alone are the source of sugar formation in ordinary dough, and the greater their number the higher is the maltose figure.

The microscopic observations recorded suggest that the characteristic mode of damage resulting in "ghosts" consists of a completely peripheral rupture or cleavage between upper and lower dish-shaped skins of the initially sound lenticular granules. The crude diagrammatic illustration is as follows:  becomes . The material of the granule divides into two superimposed portions. That the effect is unique in the completeness with which the interior of the granule is opened up to the entry of water and solutions is shown by the staining behavior.<sup>8</sup>

Where the staining reagent can enter freely the diastatic enzymes can penetrate and the products of their action can be removed outwards through diffusion. The observations described on starch to which malt extract has been added, and on a flour preparation which was rich in diastatic enzymes, leave no doubt as to this conclusion. The "ghosts," and only the "ghosts," were seen to disappear entirely. On the other hand, with a flour which was relatively poor in diastatic activity the "ghosts" persisted over a long period.

It will be realized that flours are able to behave in the accustomed way during fermentation only because of the fortuitous circumstance that they all contain some mechanically damaged starch, *i.e.* "ghosts." Flours, however, vary greatly in their content of "ghosts" and it has not perhaps been generally realized how fundamentally this consideration affects the whole question of the fermentation and gassing power of flour. Content of damaged starch and amount of enzymes are factors which are entirely independent but which together determine the diastatic activity of a flour as ordinarily observed.

### Diastatic Activity and Particle Size

From his observations the writer thinks that any factor other than the two discussed above, such as the question sometimes put forward

<sup>8</sup> Congo red is taken up by the inner starch but not by the skin. As soon as the latter is ruptured in the manner suggested, the stain enters at once uniformly from all points round the periphery. That the entry, and exit too, of the stain and of water is free, and that the inner starch is readily dissolved out of the "ghost" are shown by the behaviour described under repeated irrigation and re-staining: in the end the intensity with which the stain is taken up decreases, suggesting that much of the inner starch, which alone stains, has been removed. The behaviour with iodine is even more significant because it is well known that different members of the starch family show different colours with iodine. The soluble, inner starch gives a pure blue; the skin or envelope, in the iodine concentrations used by us, colours mauve.

of mechanical accessibility of the starch granules in bulk (whether or not they are freed from the gluten meshwork), is practically insignificant in effect beside the two main factors here postulated. Certainly it will become sufficiently clear, from observations to be described, that granularity, or particle size, of flour and milling stocks is not for practical purposes a factor determining diastatic activity. The maltose figure obtained through autolysing a suspension, even of coarse endosperm particles like coarse semolina, for a given period may be taken as substantially equal to that which would be obtained were it possible to reduce the particles to fine flour without further starch damage, and then to autolyse this flour under similar conditions.

### Distribution of Diastatic Enzymes in the Endosperm

This statement logically has an important consequence; or, rather, one might say that it depends for its validity on a fact which it is important to establish—namely, that the diastatic agent responsible for the attack on the “ghosts” in ordinary non-germinated material is uniformly distributed through the endosperm. If so of course it is easy to understand that particle size *per se* does not matter. It will not be a question of an enzyme (*i.e.*, a colloidal substance) having to diffuse into the interior of a conglomerate particle in suspension. The enzyme will be there already, uniformly in all parts of the conglomerate, merely waiting for the comparatively easy and rapid penetration of moisture, only, to come into activity.

That this is so is established beyond reasonable doubt through the following experiments.

A sample of well purified high-grade semolina was obtained. It was so prepared that it may be regarded as representing predominantly the inner portion of the endosperm, contaminated to the minimum possible extent with particles of bran coat, germ, and the endosperm from nearest the bran. Samples of it were reduced to flour in the laboratory mill in three different ways.

A mixture of 75% of this high-grade semolina was then prepared with 25% of a fifth-break (last-break) semolina prepared from a mixture of wheats. This last-break semolina was of course heavily contaminated with bran snips, pieces of germ, and generally with particles of endosperm scraped off the bran coats. The 75%-25% mixture was then reduced in the three ways comparably with the work done on the high-grade stock. The respective flours were compared. The details are given in Table I. It should be explained that the semolina (over 60 g.g.) was reduced several successive times until it afforded (1) rejected semolina offal or semolina “tails,” (2) dunst (through 60 g.g.

over 10 s.), and (3) semolina flour (through 10 s.). The dust was then taken and rolled repeatedly until a satisfactory finish was obtained, the products being "dunst offal" and dust flour. All weights recorded are on the basis of 1.5 Kg. of semolina fed to the rolls.

TABLE I

THE MALTOSE FIGURES AND ASH CONTENTS OF FLOURS MADE BY REDUCING, UNDER VARIOUS CONDITIONS, SEMOLINAS AND DUNSTS

1. Of high purity (representing chiefly the inner portions of the endosperm).
2. Mixed with 25% of offally stock (containing pieces of bran, germ and endosperm from near the bran coat).

Test No.	Conditions of reductions	Semolina reduction				Dunst reduction			
		Wt. of semo. flour	No. of rollings of semo. re-quired	Ash content of semo. flour	Malt-ose fig. of semo. flour	Wt. of dunst flour	No. of rollings of dunst re-quired	Ash content of dunst flour	Malt-ose fig. of dunst flour
Using high grade semolina									
1 A	Fast running rolls and heavy pressures.....	416	4	0.412	2.1	985	6	0.342	1.9
1 C	Fast running and light pressures.....	363	7	0.436	1.9	1048	10	0.324	1.7
1 F	Slow running and light pressures.....	345	8	0.448	1.8	1030	9	0.348	1.8
Using 75% high grade + 25% last break semolina									
2 A	As for 1 A.....	376	4	0.560	2.0	949	6	0.404	1.9
2 C	As for 1 C.....	328	7	0.628	2.0	956	10	0.414	1.8
2 F	As for 1 F.....	353	8	0.580	1.7	897	9	0.412	1.7

The flours of the second series were reduced under rolling conditions comparable with those of the first, and it can be taken that respective pairs of flours (*e.g.* 1A and 2A, 1C and 2C, and so on) have similar contents of damaged starch. Those of the second series, however, are considerably richer in endosperm material from near the bran and more contaminated with finely comminuted bran and germ—to an extent which can be judged from the markedly higher ash contents of the second series. Despite this, *the maltose figures of the flours of the second series are substantially no different, respectively, from those of the first.* That is to say, the introduction of bran, germ, and a different portion of the endosperm in significant amounts has made no difference to the extent of sugar production in one hour from a given proportion of damaged starch. Had even a small amount of diastase been added to any of these flour suspensions we know well that the sugar production in one hour would have been appreciably raised.

The writer submits the conclusion therefore that the diastatic agent responsible for sugar production in flour from normal grain is uniformly distributed throughout the endosperm.

### Maltose Figure as an Index of Content of "Ghosts"

On the foregoing basis of course, *with a given wheat sample* and over the range of maltose figures ordinarily encountered in technical practice, the maltose figure may be taken as a strict measure of the quantity of "ghosts" made through any particular milling process. This is very convenient<sup>9</sup> and has in the writer's opinion amply proved itself in extensive investigations he and his colleagues have made on the interplay of milling factors. In the present work the comparative maltose figures given will be taken as measuring comparative amounts of starch damage.

At the risk of repetition perhaps it may be mentioned here that in the case of large particles of endosperm, as in the coarse semolina fresh from the break system, the very low maltose figures usually observed are not low because of the large size of the particles. They are low because the proportion of "ghosts" is very small.

### Damaged Starch in Primary Stocks and in Grinds from Reduction Rolls

One must sharply distinguish between stocks straight from the breaks (what may be called virgin or primary material) and stock which has, once at least, passed through reduction rolls, however gently set.

TABLE II

THE TREND OF MALTOSE FIGURE WITH PARTICLE SIZE IN PRIMARY STOCKS  
(UNREDUCED STOCKS STRAIGHT FROM THE BREAK SYSTEM)

	Through 24 g.g., over 1 silk	Through 1 s., over 5	Through 5 s., over 10 s.	Flour (through 10 s.)
Corresponding range in sieve apertures ( <i>mm.</i> ) . . . .	0.86-0.39	0.39-0.27	0.27-0.135	—
Maltose figures: set				
No. 1 . . . . .	0.45%	0.41	0.62	1.39
No. 2 . . . . .	0.39	0.39	0.54	1.38

In the case of primary stocks of different particle size there is little or no tendency for maltose figure to rise as the particles become finer, at any rate down to about No. 10 silk. This is exemplified by the figures in Table II obtained on two sets of stocks sifted from two lots of purified commercial semolina made from the same wheat.

<sup>9</sup> It saves the need for direct estimation of the proportion of "ghosts" which, as will be shown in a later communication, if attempted on washed-out starch (as by Pulkki) may give rise to misleading conclusions.



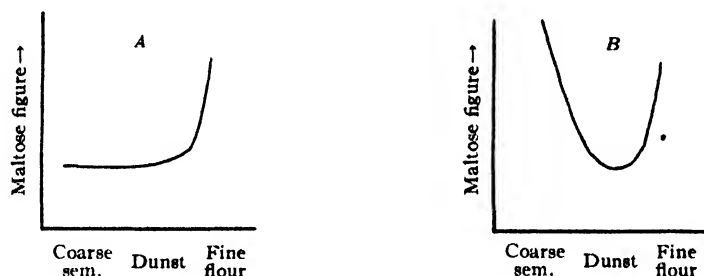
When coarse primary particles are passed through reduction rolls once, even very gently, the particles of different sizes show a characteristically different series of maltose figures. For example the mixture of the above-mentioned primary stock through 24 g.g. over No. 1 silk, in sets No. 1 and No. 2, was reduced once very gently between smooth rolls. The grind was sifted and the maltose figures of the collected respective fractions are shown in Table III.

TABLE III

THE TREND OF MALTOSE FIGURE WITH PARTICLE SIZE IN A-GRIND IN THE CASE OF A GENTLE REDUCTION OF SEMOLINA BETWEEN SMOOTH ROLLS

Through 24 g.g., over 5s.	Through 5s., over 10s.	Flour (through 10 s.)
1.28%	0.61%	1.32%

Quite generally the maltose relationships of primary stocks of different particle sizes on the one hand and of stocks produced by the partial reduction of coarse primary particles on the other may be represented as follows:



### Damage Due to "Internal" and "Surface" Factors

In seeking to understand the important general relationship described above it is necessary to distinguish between two factors at work on any given particle of stock in any reduction process. Each factor produces the same form of starch damage—"ghosts"—but in different ways. They may conveniently be called the "internal factor" and the "surface factor." To remove ambiguity let us state that the surface factor involves consideration *both* of surface of the particle and of surface of the roll. It is the shearing or scraping effect on the surface of the particle by the surface of the roll that produces a certain proportion of "ghosts" in the flour made by the scraping, the proportion produced being characteristic of the type of roll surface.

If we have "virgin" particles of semolina and subject these to some process involving gentle attrition with a minimum of pressure, such

as the gentle attrition which takes place in spouts and elevators, the whole of the flour produced is from the surface of the particles. In such a case the maltose figure of the flour produced is fairly similar to, or very slightly higher than, that produced by smooth rolls working very gently on the same semolina.

Thus the flours of Table II are really "attrition flours," the maltose figures being of the same order as that of the flour made by smooth-roll reduction of the same parent stock (coarse semolina) in Table III.

When uniformly coarse particles of endosperm are rolled it must be assumed that all sustain first a surface effect, some flour and perhaps some dust being removed from their surfaces. It may be assumed too, though it is not highly material to the present argument, that some of the coarse particles will then offer little resistance to the approach of the nip, but will crumple up on light impact into flour and much stock of intermediate sizes, especially dust. Such products will have "come off lightest."

#### The Internal Factor

The important point is that others of the coarse particles offer more resistance and are not disintegrated but are merely more or less flattened. Such particles have resisted for the moment material reduction in size, but they have undergone severe strain, the strain being the greater the greater their size. The results of the strain are shown in two ways:

- (1) A mechanical weakening of the structure of the particle so that subsequent disintegration is easier;
- (2) The conversion into "ghosts" of the whole of the starch granules within more or less well-defined zones, or rather probably planes, within the particle.

This latter is the *internal factor* in the production of starch damage during the first rolling of semolina. It takes place completely within the particle, away from any possible direct influence of roll surface, as a result of the internal shearing strain due to pressure in the organized structure of the particle. The production of "ghosts" in this way is greater the greater the work done on the particle, *i.e.* the larger and the more resistant the particle and the heavier the pressure used. Naturally therefore the resultant increase in maltose figure of the coarser stuff is found to be independent of the character of roll surface when "smooth" rolls are used, but dependent on degree of pressure employed.

The effect in practice may be well exemplified by the results of Table IV, which show the compositions and character of various grinds of a given semolina (A-roll feed) on smooth rolls. The untreated

TABLE IV

THE PERCENTAGE AMOUNTS AND MALTOSE FIGURES (SHOWN IN BRACKETS) OF THE SIEVE-SEPARATED FRACTIONS FROM GENTLE, MEDIUM, AND HEAVY GRINDS, RESPECTIVELY, OF SEMOLINA (A-REDUCTION) WITH SMOOTH ROLLS  
The Maltose Figures of Corresponding Fractions under Different Grinding Pressures Reflect the Operation of the Internal Factor

	Percent of sample over					Flour (through 10 s.)
	24 g.g.	32 g.g.	1 s.	5 s.	10 s.	
Corresponding size of sieve aperture (mm.)	0.860	0.605	0.390	0.270	0.135	—
Feed ("primary" semo.)	—	16.0	73.0	8.8	1.0	1.0
<i>Test No. 1</i>						
Gentle grind (low re- lease with smooth rolls)	—	2.3	15.2 (0.78)	24.5 (0.57)	42.0 (0.53)	15.8 (0.98)
<i>Test No. 2</i>						
Rather heavier	—	1.2	8.4 (1.19)	14.8 (0.81)	51.1 (0.64)	24.7 (0.93)
<i>Test No. 3</i>						
Very heavy	1.7	4.5 (3.3)	8.8 (2.14)	11.8 (1.36)	33.1 (0.89)	39.6 (1.02)

semolina had maltose figure 0.37%. The maltose figures of the fractions of the grinds are shown in brackets beneath the corresponding figures for granulation as shown by the sieving analysis.

It will be seen that from this given sample of primary semolina (of maltose figure 0.37%) a series of products with maltose figures ranging from 0.53% to 3.3% may be produced through one rolling with smooth rolls. The immediate point is that the maltose figures of the coarser fractions of the grinds ascend rapidly as the pressure (and flour release) is increased, and the coarser they are the more they are affected in this way. For example, with the fraction through 32 g.g. over 1s., the "internal factor" has operated markedly in the case of the very heavy grind of Test No. 3, comparatively little in the light grind of Test No. 1. Put in another way we may enunciate the principle that when the reduction roll pressure is light the proportion of the total starch damage made which is borne by the coarser particles is low; as the roll pressure is increased the proportion borne by the coarse particles increases rapidly.

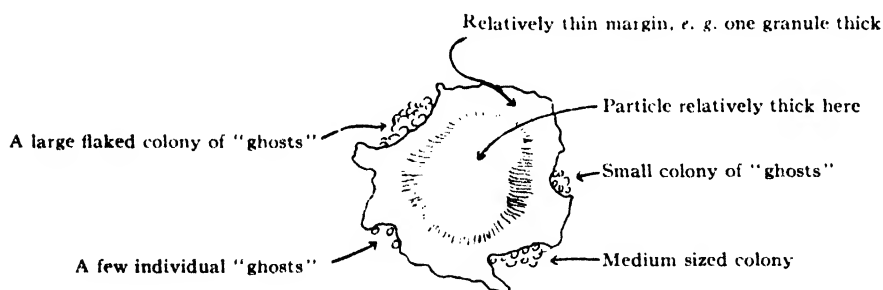
The operation of the internal factor in the way described may be confirmed microscopically.

#### The Internal Factor: Microscopical Demonstration

Generally speaking the examination of, and formation of conclusions regarding, large particles of endosperm is not straightforward because

the thickness of the mass prevents the penetration into the interior of stain (which is seized by the outermost gluten), cuts down the light for photographic purposes, and of course the occurrence of granules etc. at different depths interferes with a clear view of those above and below. The difficulty can in effect be overcome through a process of gradually and progressively dispersing or disintegrating the particle, the process being carefully and ceaselessly watched as it proceeds under the microscope. The dispersal is effected through a gentle to-and-fro motion of the cover slip over the slide with an absolute minimum of pressure.

The following observations were made on coarser particles from a smooth-roll grind somewhat similar to those through 32 g.g. over 1 s. of Test No. 2 in Table IV. The maltose figure of the feed in this case was 0.56 and that of the coarse particles in question in the grind 1.50. For convenience we will speak of "feed particle" and "grind particle" as meaning particles of the same nominal size taken from the feed and grind respectively. The grind particles are all more or less flattened though to very different extents according to their size. If the moderately flattened, discrete particles which represent the major part of the endosperm present are viewed after a short soaking in the stain, without any mechanical disarrangement, the appearance is much as shown diagrammatically in the sketch:



It is noteworthy that what external damaged starch there is occurs at the edges of such particles and not on the upper or lower flattened surfaces of the thicker central part.

The actual appearance of such a particle is shown in Plate 6A and that of the feed particle (of maltose figure 0.56) in Plate 6B. No very great difference is apparent in this state of the particles apart from the presence of peripheral "ghosts" in Plate 6A and the contouring, a particle of unrolled semolina being thick throughout without any thinner margin.

The case is very different, however, when the cover slip is gently worked so as to cause gradual disintegration of the particle. The

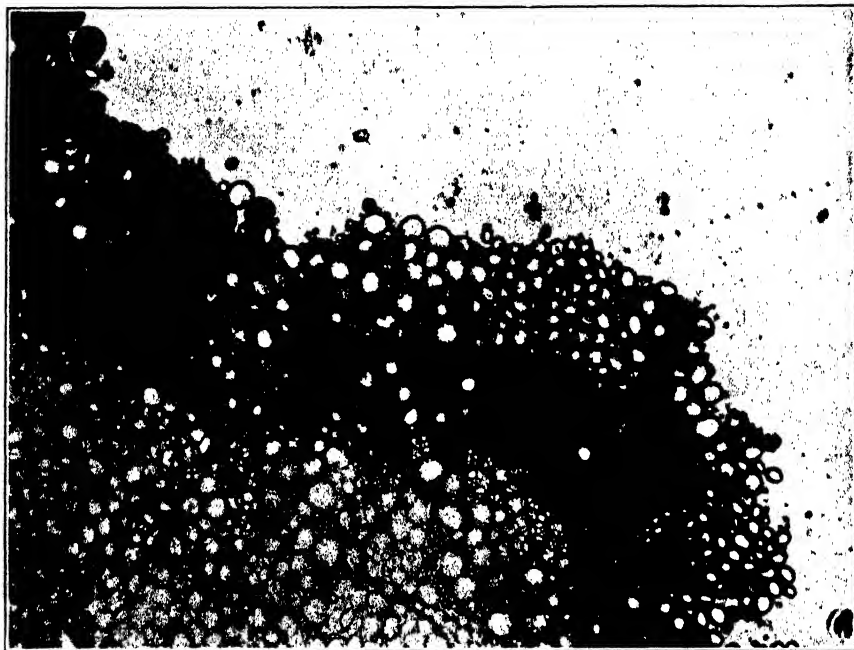


PLATE 6A

A grind particle (stained with congo red).

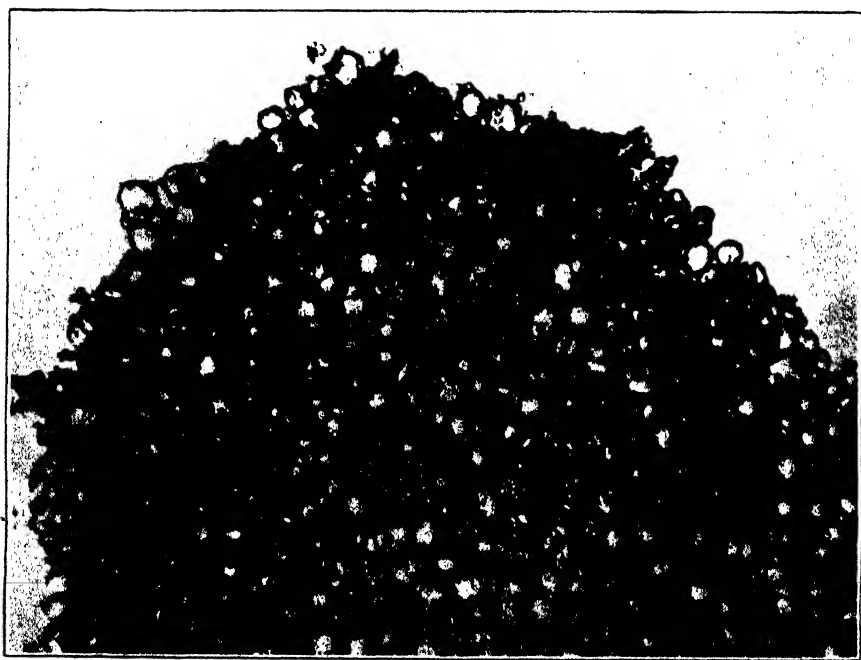


PLATE 6B

A feed particle (stained with congo red).

starch granules are gradually freed from the delicate gluten meshwork, teem towards the outside and are dispersed in the surrounding liquid. (Incidentally the "ghosts" behave somewhat differently in this respect from the sound granules as has been mentioned earlier. For instance, in working a particle a patch of "ghosts" hung together astonishingly well whilst the neighboring sound granules were washed freely away.) The gluten in turn consolidates itself forming very tangible rubbery rolls which are gradually freed from starch granules.

An idea of the true state of the starch in the interior of the grind and the feed particles, respectively, is given by Plates 7A and 7B. The large patches of apparently dark material in both photographs are lumps of deeply stained gluten. The starch in process of being dispersed in Plate 7B from the particle of original semolina is entirely sound. The process has been carried to an advanced stage in the case of the subject of Plate 7A; here the grind particle has been practically completely disintegrated, the lumps of gluten being relatively free from starch, and all the granules in the field were originally well in the interior of the particle. Many "ghosts" are to be seen amongst the sound starch granules. The important thing, however, is the constant watching of the process from the beginning of gentle dispersion onwards, the result leaving no doubt that, apart from the "ghosts" seen on the edges of the particle at the start, all those in the field of view *are from zones in the interior of the particle*. To make this clear it must be pointed out that when even strong stain solution is used in making up the slide, the stain is absorbed by the "ghosts" situated at the edges described and by the gluten of the outer layer all round the particle so that only water penetrates to the interior. On working the cover slip, the starch granules constantly streaming out from the interior of the particle include periodically batches of "ghosts" *which are unstained*, having been in contact with water only in the interior of the particle. Only when they reach the dye present in the liquid surrounding the particle do they at once take up the stain.

We are fortunate in being able to illustrate well this rather "tricky" matter through having secured a clear photo at a critical moment in the early stages of the dispersal of a fairly flattened grind particle. In Plate 8A there is a thin strand of gluten running in a diagonal direction which formed quickly and served admirably to protect the starch granules coming from the interior (situated towards the top and left-hand corner) from the stain in the outer liquid encroaching from the lower right-hand corner. To the left of the strand may be seen several "ghosts" as yet unstained, some of which are in a small colony which has moved out boldly amongst the freely scattering

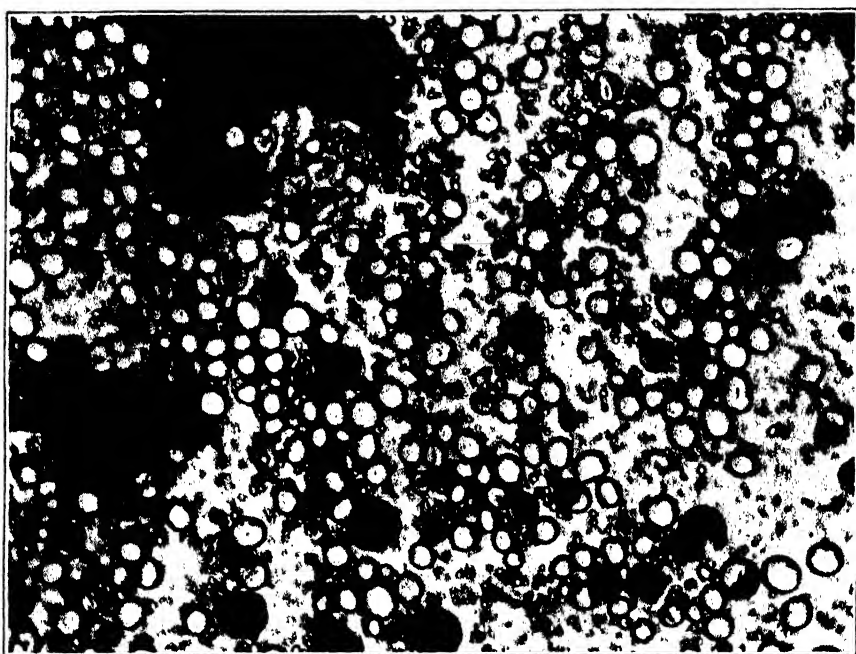


PLATE 7A

The grind particle after completion of "working" (stained with congo red).

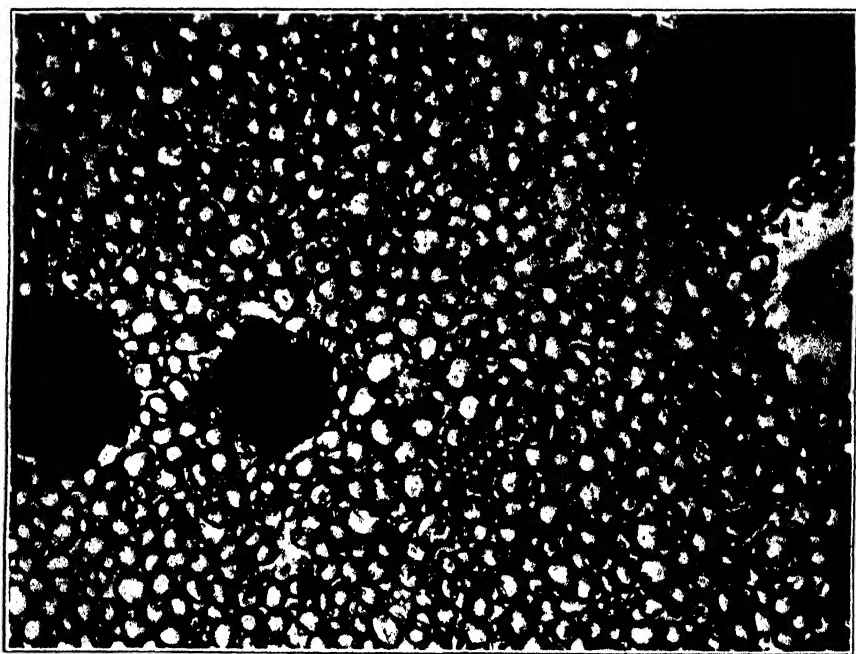


PLATE 7B

The feed particle after completion of "working" (stained with congo red).

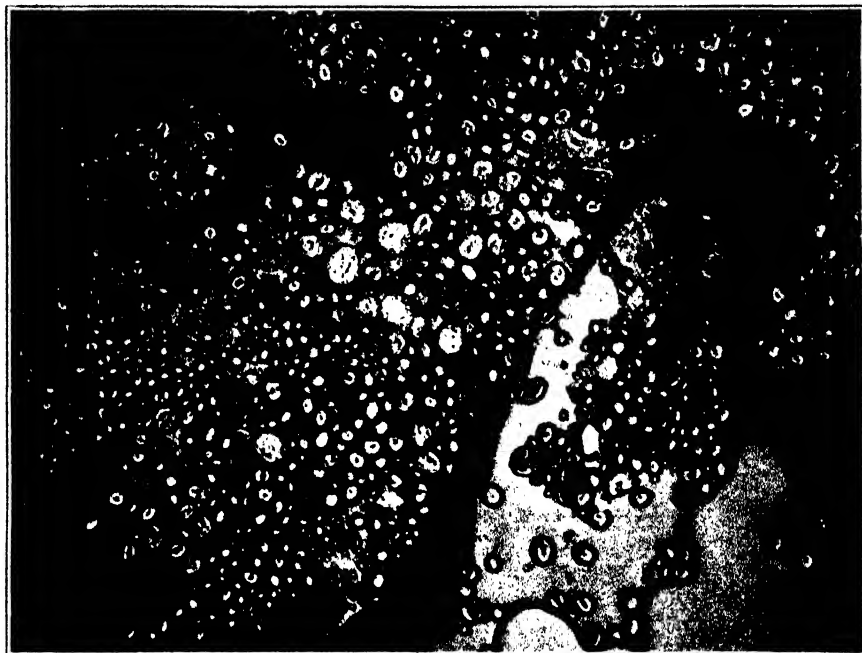


PLATE 8A

A grind particle after a little "working" (stained with congo red).

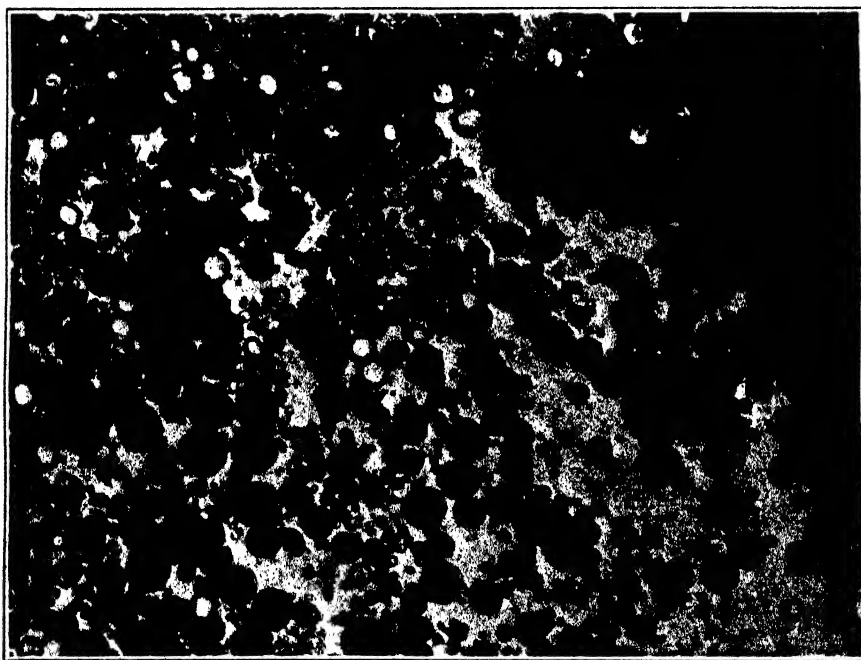


PLATE 8B

A particle of tail stock grind after "working" (stained with congo red).



sound granules. In marked contrast there is a number of stained "ghosts" on the right-hand side of the strand which initially were attached to the edge of the particle.

### High Maltose Figures in Low-Grade Reduction Flours and "Tail Stocks"

If grind particles constitute a feed passing through smooth rolls a second time, any that escape disintegration will be very much flattened and will carry very severe internal damage. In this way the effect of the "internal factor" is cumulative, the maltose figure of such particles increasing markedly after each rolling.

With such particles there is no difficulty microscopically in obtaining evidence of the damage. They are really large flakes and are relatively thin so that the stain is able to penetrate fairly readily. On disintegrating a little the appearance is very striking as is shown by Plate 8B, which shows the case of coarse particles from a second consecutive grind with smooth rolls, the maltose figure being 4.3%. Very few of the larger granules are present except as "ghosts."

The practical consequence of this phenomenon is not difficult to understand. When coarse semolina is reduced in practice (on *A* reduction) the tails of the grind pass on to rolls further down the reduction system where they are again rolled. Now it is worth considering briefly the effect of further reduction on particles which have already been subjected to the "internal factor" as a result of having once been rolled. The maltose figure of the products from the second rolling (*i.e.*, the reduction of "tail stock") is a resultant of the following: (a) the breaking up to a greater or less extent of strained, damaged zones, *i.e.*, of colonies of "ghosts," from the interior of the large particles; (b) the action of the surface factor in which as before different types of roll surface behave differently and characteristically, but which is less than in the case of primary semolina owing to the mechanical weakening, already referred to, of the structure of the particle. That is to say, less resistance is offered to the scraping off of flour.

There is no need here to go into detail regarding the interplay of these factors under different conditions of reduction, but it may be stated that the effect usually is as follows: The flour formed is no lower than the feed in maltose figure and may be rather higher. The dunst is always appreciably higher in maltose figure than the flour, a fact which differentiates the operation from the reduction of primary semolina where the dunst tends to be appreciably lower in maltose figure than the flour (*A* roll). The difference is occasioned by the tendency of the "ghosts" liberated from the interior of the large particles to

adhere and remain together in comparatively large colonies. Relatively far more of the damaged starch from this source therefore remains in the dust than is able to get through the flour silk.

Since in the reduction of "tail stock" the feed is high in maltose figure, the flour released is necessarily high also. This is the only explanation of the high maltose figures frequently found in very low-grade flours—contrary to the opinion sometimes expressed that such flours are high in maltose figure because heavily contaminated with offal particles or because they are very fine. Moreover, if the grinding pressure is increased on the head rolls of the reduction system the effect will at once be to raise the maltose figures of certain flours made further down the mill because under such conditions the maltose figure of the head-roll tail stock is greatly raised and therefore, also, the feed to the later reductions.

By way of partly summing up, it is possible to enunciate the following principles without which fluctuations of maltose figure, to be observed in the course of the milling process, are unintelligible:

1. Intermediate reduction stock carries damaged starch which becomes liberated during a subsequent release of flour.

2. The damaged starch can be carried in this way to varying degrees. Therefore, in the reduction of dust, for example, quite similar conditions of rolling may give rise to flour of appreciably different maltose figures, according to the previous history of the feed. Thus dust from *A* reduction with smooth rolls and moderate pressures may commonly have a maltose figure of 0.7%. If on the other hand rougher rolls are used and also rather heavier pressures the figure will commonly become 1.0% or even rather higher. Since this stock is a major constituent of the feed to *C* roll there will commonly be a difference in the two *C* feeds of approximately 0.25% in maltose figure. The writer has found as a convenient rule in such cases that, other things being equal (identical operation of the *C* roll), the difference may be reckoned as added to the maltose figure of the flour made during the dust reduction. If the conditions are such that in the first case the *C* flour has 1.4% maltose figure, then in the second case it will have 1.65% maltose figure and so on. These observations lead, together with the considerations of the "internal factor" already discussed, to the third principle.

3. The maltose figures of flours made at various points throughout the reduction system are influenced through changes made in the head reductions.

It is interesting now to consider the implications of the theory of the two factors in regard to the conditions of operation of the coarse

semolina reduction (*A* rolls), and to see how it fits with what is found in practice.

### The Surface Factor

The surface factor—the scraping or attritional factor—should obviously be dependent for its extent on the roughness of the roll surfaces and on the differential speed with which they are driven. One would expect these to affect considerably the proportion of “ghosts” in the flour which consists mostly of the stuff torn off the surfaces of the large particles. One would expect them to affect the dust liberated to a much smaller effect because the extent of surface produced is so much less in relation to mass than in the case of the flour. One would not expect them to affect appreciably the maltose figure of the coarser fractions of the grind because the starch damage here is chiefly in internal zones as a result of compression.

That these expectations fit in with practice will be seen from the results of comparative reduction tests on a sample of coarse semolina (*A*-roll feed) given in Table V. Maltose figures are shown in brackets beneath the corresponding figures for granulation according to the sifting analysis.

The very great effect of the matt rolls at the higher differential on the maltose figure of the *A* flour will particularly be noticed. Gen-

TABLE V

THE MALTOSE FIGURES (SHOWN IN BRACKETS BENEATH THE CORRESPONDING FIGURES FOR PERCENTAGE COMPOSITION AS SHOWN BY SIFTING ANALYSIS) OF THE FLOUR AND OTHER FRACTIONS OF THE GRIND OF SEMOLINA (*A*-REDUCTION) MADE WITH SMOOTH ROLLS, AND WITH VERY COARSELY MATT-SURFACED ROLLS AT DIFFERENT DIFFERENTIAL SPEEDS

	Percent of sample over					Flour (through 10 s.)
	24 g.g.	32 g.g.	1 s.	5 s.	10 s.	
Corresponding sizes of sieve apertures ( <i>mm.</i> )..	0.86	0.605	0.39	0.27	0.135	—
Feed (untreated semolina).....	12.4	44.3	37.3	0.5	3.5	2.0
Grind with smooth rolls 1.27/1 differential.....	3.3	9.3 (2.12)	16.0 (1.49)	16.1 (1.00)	35.6 (0.85)	19.0 (1.40)
Grind with very coarse matt rolls at 1.27/1 differential.....	1.0	8.0 (1.72)	19.8 (1.21)	15.1 (0.90)	35.0 (0.89)	20.9 (2.34)
Grind with very coarse matt rolls at 2.1/1 differential.....	1.3	8.0 (1.74)	21.5 (1.12)	14.2 (0.95)	35.3 (0.98)	19.9 (3.93)

erally speaking the effect of differential is the more marked the rougher the roll surface and, as might be expected, it is progressively greater the greater the differential.

The very coarsest fractions of the grind tend to be lower in maltose figure with very rough rolls because a given release of flour can be made with rather less roll pressure owing to the greater attritional power of the surface. There is therefore less flattening of the coarsest parts of the grind and smaller production of internal damage.

### Effects of Increase in Grinding Pressure in the Reduction of Coarse and Fine Particles

Returning now to consideration of the "internal factor" the data of Table IV have already shown sufficiently well how, as regards the coarser fractions of the grind, the use of increased roll pressure produces markedly increased starch damage in the expected way. What however is the effect on the maltose figure of the *A* flour of increasing the *A* roll pressure? It is strikingly characteristic of the reduction of coarse semolina particles that the maltose figure of the *A*-roll flour is substantially unchanged through variation in rolling pressure. This is shown well in Table IV. It is equally characteristic of this reduction that the *A*-roll flour is not made finer in granulation through increasing the rolling pressure; in fact it tends to be made grittier and larger in average particle size. This will be explained presently (see next section).

These considerations apply to the reduction of *coarse* endosperm particles. There are significant differences in the reduction of *finer* particles such as middlings or dunst, for example *C*-roll feed.

In the reduction of middlings the effect on starch damage of variation in differential roll speed is almost negligible compared with that in the reduction of coarse particles but the effect of the nature of the roll surface *per se* is still appreciable though somewhat diminished. It is in the effect of roll pressure that the reduction of fine stock is most sharply differentiated from that of coarse.

Table VI shows the effect on maltose figures (shown in brackets) of the fractions of the grinds produced by rolling middlings with smooth rolls (at 1.27/1 differential) set to various pressures. The maltose figure of the feed as a whole was 0.61%.

It should be explained that a thorough mechanical sifting test is used to analyze the samples and that the release of flour described as high in the table (test No. 74) is actually no more than is often found in commercial practice with *C* reduction. The small percentage found over 1 s. under these conditions represents flake production which always occurs to some extent when smooth rolls operate on fine stocks

TABLE VI

PERCENTAGE AMOUNTS AND MALTOSE FIGURES (IN BRACKETS) OF THE SIEVE-SEPARATED FRACTIONS FROM GENTLE, MEDIUM, FAIRLY HEAVY AND HEAVY GRINDS, RESPECTIVELY, OF MIDDINGS (C-REDUCTION) WITH SMOOTH ROLLS  
The Trend of the Figures Is to Be Compared with That of Table IV (Dealing with Semolina)

		Percent of sample over			Flour (through 10 s.)
		1 s.	5 s.	10 s.	
Corresponding sizes of sieve apertures (mm.) . . . . .		0.390	0.270	0.135	—
Test No.	Conditions of rolling				
—	Feed . . . . .	—	11.0	76.0	13.0
71	Low release . . . . .	—	4.4 (1.33)	71.0 (0.67)	24.9 (0.82)
70	Medium release . . . . .	—	3.4	46.0 (0.85)	50.3 (0.96)
72	Fairly high release . . . . .	1.0	3.1	29.1 (1.24)	66.7 (1.12)
74	High release . . . . .	1.0	5.8	23.9 (1.78)	70.0 (1.26)

at high pressures. In fact under such conditions the dunst particles in the grind are flat and they and many of the flour particles are simply flakelets of varying size.

As seen from Table VI, in the reduction of fine particles at low releases (gentle pressures) with smooth rolls there is very little damage done to the starch. The increment in maltose figure (difference between that of the feed and that of the particular fraction of the grind) is small in the case of the flour. This is a marked differentiation from the behavior of coarse particles where, even with the gentlest reduction using smooth rolls, the increment in the case of flour is large (say 0.7) and in fact as high as under conditions of severe reduction.

Further, with fine particles, the maltose figure of the flour rises progressively as the pressure and release are increased. The rise becomes more rapid as the release increases.

The maltose figure of the remainder of the grind, chiefly the dunst, also rises even more rapidly. The "increment" in the case of the dunst is practically non-existent at low releases but it greatly exceeds that for the flour at high releases.

How are these differences between the reductions of coarse and fine particles to be explained? It should be noted that reduction of middlings differs markedly from that of coarse semolina in that increasing rolling pressure affects the granulation of the flour. The flour which is coarsely granular at lower releases becomes progressively and markedly finer as the release is increased.

The radical difference between the effect of varying pressure on the maltose figure of the flour made during a rolling of coarse stock on the one hand, and of fine stock on the other, must not, however, be attributed simply to a consequence of the constant granularity in the one case and the increasing fineness of the flour in the other. It is due, rather, to the different underlying factors which in turn give rise to the different effects on granularity.

### **Interpretation of the Different Effects Observed in Reduction of Coarse and Fine Particles**

It appears that the essential features of the reduction of milling stocks may be viewed in this way: a particle of coarse semolina may be, let us say to simplify matters, roughly cubical in shape and, say, 0.5 mm. long each side. On crushing under normal conditions as in *A* reduction it may break up principally into, say, 16 pieces, the ratio of whose length of side to that of the original particle will, of course, be the cube root of 1/16, namely, 1/2.5. That is to say, the new pieces will have a length of side on the average of 0.2 mm., which would correspond to stock overtailling a 10 silk but passing through a 60 g.g. Now if the pressure on the rolls is considerably increased, *i.e.* the grind made lower, the original particle will break up not into 16 but principally, say, into 32 fragments, so that the average size of the particle formed has moved further down. In addition to this sort of process, in *A* reduction there is always a certain amount of quite fine flour produced which must be regarded as scraped or torn off the surface of the large particle through contact with the roll surface, under the influence of the differential speed.

When the grind is lowered and the pressure increased in the case of *A* reduction the original flour is thus reinforced by a considerable amount of relatively coarse particles, which are dust as it were moved or reduced a stage down. In other words, the increased pressure has caused many of the particles of the chief product, the dust, to be smaller than they were in the first place: not very much finer, but fine enough now to pass through the flour silk. It is in this way that the, at first sight, surprising result is obtained that increasing the pressure on *A* roll, so that the release of *A* flour increases from, say, 18% to, say, 35% or even more, does not cause the *A* flour to be at all finer and softer but may even make it feel more gritty. What is the effect of the increased pressure on maltose figure of the *A* roll flour? It has been briefly mentioned already how the dust from *A*-roll grind, the stuff of intermediate granularity, has the lowest maltose figure of all parts of the grind. Its particles largely escape the internal damage (production of "ghosts" in zones inside the particle) which happens

to the coarser overtails. They do not receive such severe peripheral damage as the fine flour produced through the action of the roll surface in tearing material away from the surface of the large particles. At the same time, any peripheral damage which they do receive has much less effect on the maltose figure, because, owing to the greater particle size compared with the flour, and lower ratio of surface area to total mass, the damage per unit weight is so much less. Much the same applies to the material now under discussion which, when the pressure on *A* rolls is increased, becomes in effect dust which is just fine enough to pass through the flour silk. The amount of damaged starch it brings into the flour is small enough in relation to the total amount of sound starch introduced not to cause any increase in maltose figure.

It is possible too that when increased roll pressure is applied to *A*-roll feed, a certain amount of the finer material liberated originates directly from the interior of the large particles of endosperm constituting the bulk of the semolina, and as such has not been influenced at all by the surface action largely affecting the flour made under conditions of lighter rolling. Either of these factors ensures that the maltose figure of the *A*-roll flour does not increase upon lower grinding.

In *C* reduction one starts in a much lower octave, as it were. The feed as a whole is relatively very near in particle size to the mesh apertures of the flour silk, and when any given particle breaks up on high grinding into a certain limited number of smaller pieces the whole of these pass through, say, a 10 silk, the flour so formed being quite coarse and granular.

As the grind of *C* roll is lowered, and the release made greater, the bulk of the particles formed become progressively and relatively fairly uniformly smaller in size. The *C* flour becomes finer and softer to the feel. Not only so, but owing to the greater violence (greater energy expended) more and more of the starch granules situated around the surfaces of these particles become damaged (converted into "ghosts"). A good idea of the change occurring may be had from a study of Plate 9, which shows microphotographs (magnification approximately  $\times 120$  diameters) of two of the *C* flours of Table VI, in congo red stain. Photograph A shows a typical view of flour No. 71 corresponding to a release from the *C*-roll feed of only about 25%, with a maltose figure of 0.82%. Photograph B shows flour No. 74, corresponding to a 70% release, with a maltose figure of 1.26%. It will be seen how on the whole the flour particles are very considerably smaller in the latter flour. The important thing with respect to maltose figure is the presence of a greatly increased number of "ghosts" in flour No. 74.

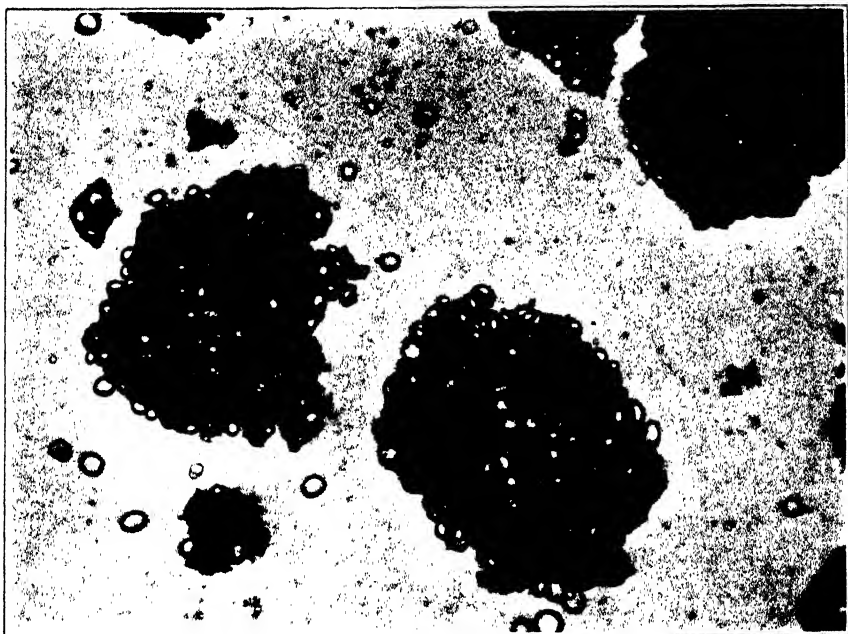


PLATE 9A

Particles of flour made by reducing middlings at different roll pressures.  
Release 25%, maltose figure 0.82.

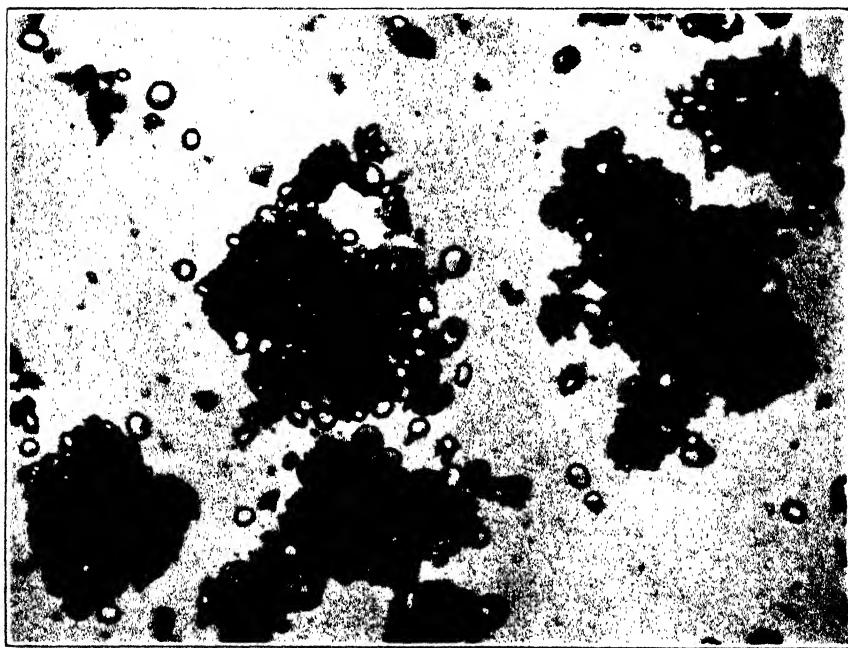


PLATE 9B

Release 70%, maltose figure 1.26.



### Production of Damaged Starch as Affected by Type and Hardness of Wheat

This paper cannot well be concluded without a brief reference to a practical consequence of the considerations given. The production of "ghosts" in the interior of large particles during reduction has been shown to be due to the strain resulting from compression. Naturally the extent of this strain will depend on type and condition of wheat. To a considerable extent this is true also of the operation of the surface factor. Generally therefore other things being equal the maltose figure of the flour becomes higher the harder the wheat. This is markedly so under conditions of severe milling because the internal factor then plays a preponderating part. In illustration Table VII may be given.

TABLE VII

MALTOSE FIGURES (SHOWN IN BRACKETS BENEATH THE CORRESPONDING FIGURES FOR PERCENTAGE COMPOSITION AS SHOWN BY SIFTING ANALYSIS) OF THE FLOUR AND OTHER FRACTIONS OF GRINDS, UNDER COMPARABLE CONDITIONS, OF SEMOLINAS FROM HARD AND FROM SOFT WHEATS

	Percent of grind over					Flour (through 10 s.)
	24 g.g.	32 g.g.	1 s.	5 s.	10 s.	
Corresponding lengths of sieve apertures (mm.) . . . . .	0.860	0.605	0.390	0.270	0.135	—
Grind of Manitoba semolina . . .	1.0	6.0 (3.67)	12.4 (2.68)	18.8 (1.74)	40.4 (1.10)	20.4 (1.53)
Grind of soft-wheat semolina . . .	1.3	4.8 (1.76)	12.6 (1.19)	17.5 (0.81)	43.6 (0.64)	20.2 (0.93)

Table VII shows the composition according to sifting analysis and (in parentheses) the maltose figures of the respective fractions of the grind in the cases of (1) a reduction of coarse semolina from all Manitoba wheat, and (2) a similar reduction of coarse semolina from soft wheat (ordinary Yeoman). The feeds (untreated semolinas) were of similar granulation.

It will be seen that, as a result of the one grind, the Manitoba semolina gave a series of products ranging from 1.10% to 3.67% in maltose figure, and the soft wheat from 0.64% to 1.76%. The range in the one case is more than double that in the other, as a result of the different degrees to which the "internal factor" operated in the two cases.

### Summary

All flours contain a certain proportion of their starch granules mechanically damaged as a result of milling processes. This is a fortuitous advantage, for in the absence of such damaged granules no

appreciable diastatic action would occur in the suspensions or doughs made from flour from ordinary sound wheats. Wheat-starch granules are subject to more than one type of mechanical damage but it is shown that only one type results from roller milling processes. The nature of this particular type of damage is discussed. Owing to their characteristic microscopical appearance in the unstained state, granules affected in this way have been dubbed "ghosts"—a not inconvenient way of avoiding a plurality of words in referring to them.

It is shown that the enzymes responsible for diastasis in sound wheat products must be regarded as uniformly distributed throughout the endosperm. Diastasis in a water suspension is not affected by particle size *per se*, and in flours and intermediate stocks variously milled from a given wheat the maltose figure is a measure of the number of "ghosts" present.

"Ghosts" are present in flours to very different extents according to the conditions of milling. The conditions under which they are produced during milling are analyzed. Two broad factors operate: the "surface factor" and the "internal factor": the one dependent on the shearing or scraping of material from particle surfaces, the other on the crushing or partial flattening of larger particles. It is characteristic of the type of damage to the granule in "ghosts" that it is produced by both the shearing and the rapid crushing action encountered in roller milling. The distinction between the two factors however is important in determining the "ghost" content (or maltose figure) of the final straight-run flour.

With primary stocks of different particle sizes (primary means coming directly from the breaks, *i.e.*, unreduced), the maltose figure remains low as the particles become finer until the flour itself (say throughs of No. 10 silk) is reached. It is shown that the operation of the "internal factor" causes the coarser fractions of a reduction roll grind to be relatively high in maltose figure, because their particles carry *internal* zones of damaged starch. These result from the strain set up in the particles as a result of the partial flattening during the rolling. When coarse primary stock (such as *A*-roll feed, in British milling terminology) has passed once through smooth rolls, the maltose figures of the coarser fractions of the grind are thus relatively high, whilst those of the intermediate fractions (dunst or *C*-roll feed) are low. The heavier the roll pressure and the coarser the particles concerned, the higher the resulting maltose figure, owing to the greater intensity of operation of the internal factor. The flour produced from such coarse particles (*A*-roll flour) has a more or less high maltose figure which however is not affected through change in grinding pressure.

Its content of "ghosts" is due to the surface factor only, and its maltose figure accordingly is markedly affected through nature of roll surface and differential roll speed, factors without influence on the internal factor, which causes the damage in the coarser fractions of the grind.

In general the important principle appears that any incompletely reduced particles (intermediate reduction stock: dunst or tails) carry damaged starch which becomes liberated, in addition to that freshly made, during a subsequent release of flour, *i.e.* when the intermediate stock in question becomes the feed to a later reduction. It is shown that this results in, and is the sole cause of, relatively very high maltose figures of flour and other stocks from later reductions, and that the magnitude of such maltose figures depends sensitively on the conditions of the earlier, or head, reductions. In general the effect of the internal factor is cumulative, the maltose figure of the coarser particles which may escape disintegration increasing markedly after each rolling.

It is explained that there are significant differences with respect to maltose figure between the reduction of finer particles (middlings or dunst, *e.g.* C-roll feed) and that of coarse (semolina, *e.g.* A-roll feed). In the reduction of middlings the effect on starch damage of variation in differential roll speed is almost negligible compared with that in the reduction of coarse particles, but the effect of the nature of the roll surface *per se* is still appreciable though somewhat diminished. It is in the effect of roll pressure that the reduction of fine stock is most sharply differentiated from that of coarse. With gentle reduction of fine stock few "ghosts" are produced, but increase in the roll pressure leads to a rapid rise in maltose figure of flour and a still more rapid rise in that of the remainder of the grind, chiefly the dunst. An explanation is advanced of the mechanism underlying the essential differences between the reduction of fine and coarse particles.

An interesting practical consequence of the considerations given is in the case of different types of wheat. The extent of operation of the internal factor depends on type and condition of wheat; to a considerable extent this is true also of the surface factor. Generally therefore the maltose figure of the flour becomes higher the harder the wheat. This is markedly so under conditions of severe milling because the internal factor then plays a preponderating part. Differences in diastatic activity between flours from different types of wheat are thus not *necessarily* due to differences in amylase content or in starch "susceptibility." They are at least partly to be attributed to differences in the physical hardness of the endosperm as affecting the extent of the damage to the starch during milling.

### Acknowledgment

The writer wishes to thank E. A. Fisher, Director of Research, for his interest and encouragement throughout this work. He is indebted to Dr. Fisher and to the Council of the Research Association of British Flour-Millers for permission to publish this paper. He acknowledges gratefully careful assistance rendered by E. N. Greer, who carried out the photographic work, and by A. Pickard, J. Thomlinson, R. W. Palmer, and G. Baker in various phases of the investigations.

### Literature Cited

- Alsberg, C. L., and Griffing, E. P.  
1925 Effect of fine grinding upon flour. *Cereal Chem.* 2: 325-344.
- Blish, M. J., Sandstedt, R. M., and Kneen, E.  
1938 The cereal amylases with reference to flour and malt behavior. *Cereal Chem.* 15: 629-658.
- Huss, H.  
1922 Untersuchungen über die Quellung der Stärkekörner. *Archiv. für botanik, Stockholm* 18: (8) 1-23.
- Pulkki, L. H.  
1938 Particle size in relation to flour characteristics and starch cells of wheat. *Cereal Chem.* 15: 749-765.
- Sandstedt, R. M., Blish, M. J., Mecham, D. K., and Bode, C. E.  
1937 Identification and measurements of factors governing diastasis in wheat flours. *Cereal Chem.* 14: 17-34.
- Stamberg, O. E., and Bailey, C. E.  
1939 Studies on wheat starch. II. The action of amylases on raw wheat starches. *Cereal Chem.* 16: 319-330.

## A BIOCHEMICAL AND TECHNOLOGICAL STUDY OF PUNJAB WHEAT VARIETIES<sup>1</sup>

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(Received for publication August 9, 1939)

Wheat is the mainstay of Punjab agriculture. The importance of Punjab as a wheat-growing country can well be imagined by the fact that of all the provinces of India, taken singly, it has the largest acreage under wheat. It not only produces enough wheat for home consumption, but is the premier wheat-exporting province. Of the total quantity of wheat exported from all India, Punjab contributes the major portion. The merits and shortcomings of Indian export wheat, whatever they may be, are largely those of the Punjab wheat.

### Area, Production, Yield

During the decennial period ending with 1934-35, out of an average of 33.2 million acres on which wheat was grown annually in India (including native states), 9.4 million acres or 28% belonged to the Pun-

<sup>1</sup> Condensed from a thesis presented by Rattan Singh to the Graduate School of the University of Minnesota in partial fulfillment of the requirements for the degree of Master of Science.



Fig. 1. Outline map of India.

TABLE I

ACREAGE AND PRODUCTION OF WHEAT IN INDIA AS COMPARED TO THAT OF THE PUNJAB, INCLUDING NATIVE STATES IN BOTH CASES

Year	Acreage—millions of acres		Production in millions of tons	
	India	Punjab	India	Punjab
1925-26	31.0	9.5	8.8	2.9
1926-27	31.8	9.4	9.1	2.9
1927-28	32.7	9.0	7.9	2.3
1928-29	32.5	10.0	8.7	3.1
1929-30	32.2	10.0	10.7	3.8
1930-31	32.7	9.3	9.5	3.1
1931-32	34.3	9.1	9.2	2.8
1932-33	33.5	8.6	9.6	2.8
1933-34	36.6	9.8	9.6	2.8
1934-35	35.0	9.0	9.9	3.0
Average	33.2	9.4	9.3	2.9
Percent of total	—	28.3	—	31.1

jab. Of these 9.4 million acres, 5.2 million or 55.3% is irrigated and 4.2 million or 44.7% is *barani* or rain-dependent.

The wheat area of the Punjab ranges annually between 9 and 10 million acres. This variation occurs in the *barani* area which, of

necessity, has to depend upon the rains. The irrigated area, however, remains almost constant from year to year (Table II).

TABLE II  
EXTENT OF IRRIGATED AND UNIRRIGATED WHEAT ACREAGE IN THE PUNJAB

Year	Wheat acreage in the Punjab, in millions of acres	
	Irrigated	Unirrigated
1925-26	5.1	4.4
1926-27	5.0	4.4
1927-28	5.1	3.9
1928-29	5.3	4.7
1929-30	5.6	4.4
1930-31	5.4	3.9
1931-32	4.9	4.2
1932-33	5.0	3.6
1933-34	5.0	4.8
1934-35	5.2	3.8
Average	5.2 (55.3%)	4.2 (47.7%)

During the same period the average annual production was 2.9 million tons, which was almost 39% of the total production of 7.5 million tons for British India. If Indian states are included, then it becomes 31% of a grand total of 9.3 million tons. A diagrammatic representation of acreage and production of wheat in the different provinces of India is shown in Figure 2.

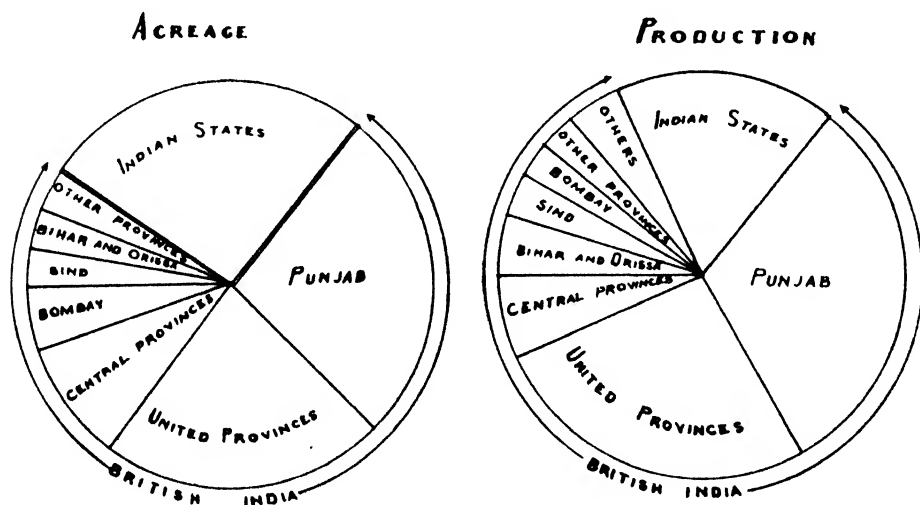


Fig. 2. Distribution of wheat production in India.

The average yield per acre of wheat in the *barani* area is estimated to range between  $9\frac{1}{4}$  and  $10\frac{3}{4}$  bushels (bushel = 60 lbs.). On the irrigated lands the yield is between 15 to 17 bushels, and the combined

average is between 12 and 14½ bushels, *i.e.*, of the order of the United States. Though the average yield of the province as a whole is low, yields of 28 to 30 bushels from large-size blocks of a thousand acres or more of land in the new canal colonies are on record.

### Internal Consumption and Trade

It has been estimated that something like 72% to 84% of the total production is retained in the province and largely consumed in the form of *chapaties* (unleavened pan-baked cakes). There are about a score of modern roller mills working in the province. A certain amount of fermented bread in the form of loaves is produced by the *bazar* bakers and other bakeries in the cities and is coming into use. The remaining 16% to 28% is exported either to other provinces, or out of India, chiefly through the port of Karachi (Figure 1) under the label of choice white Karachi or red Karachi wheat.

Figure 3 gives the per-capita consumption of wheat in the different provinces of India. This shows that in general the Northern parts of

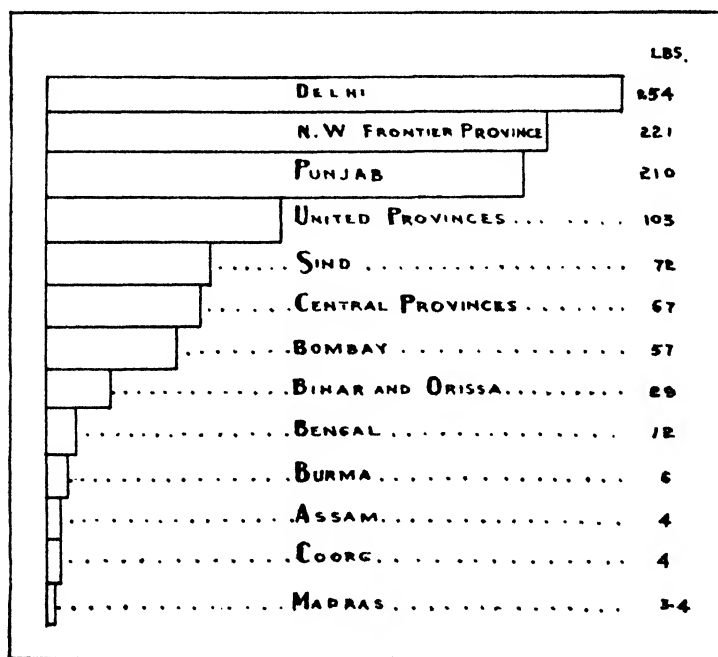


Fig. 3. Per-capita consumption of wheat in the provinces of India.

India produce more wheat and the consumption per capita is proportionately greater. This, however, declines towards the south or east where rice is the staple cereal crop. The lowest consumption per capita of wheat is in Madras.

### **Area and Location of the Punjab**

The Punjab comprises an area of 133,000 square miles, of which 36,500 square miles belongs to Indian states, and is one of the provinces of India. Mexico, southern Arizona, Texas, Mississippi, Georgia, etc., are situated in about the same latitude. Gibraltar is only two degrees further north of the equator than the northern districts of the Punjab.

Lyallpur, one of the 30 districts, is situated in the canal colony area of the Punjab. It was founded about 45 to 50 years ago when this portion of the Punjab, till then an uncultivated waste, was brought under irrigated cultivation by means of "perennial" canals. It is the headquarters of the Department of Agriculture of the Punjab as well as the seat of the Punjab Agricultural College and Research Institute.

### **Climate and Soil of the Punjab**

The Punjab is an inland province. Since it has a light soil and much of it is 500 to 600 miles from the sea, it is subjected to extreme variations of temperature. In June maximum shade temperatures have been recorded as high as 122.6°F. The recorded temperatures have been as low as 8° below the freezing point. This low temperature, however, does not do much harm because the cool mornings are usually followed by sunny days. Hot weather generally begins in February and becomes rather unpleasant in April when hot winds come from the west.

There are two well defined rainy seasons, one of which is known as the summer or monsoon and the other the winter rainy season. Monsoon season in Punjab begins about the end of June or the middle of July and ceases about the middle of September or earlier. The winter rains, from December to March, are more uniformly distributed. It is on the volume and distribution of both of these rainy seasons that the annual acreage and yield of wheat in the Punjab depend.

Of the 30 districts there are about a dozen which, being either mountainous or submountainous, have enough annual precipitation for ordinary agricultural purposes, *i.e.*, 25 inches or more. Elsewhere it is less than 25 inches and diminishes in some southwestern districts to as little as 5 to 7 inches. Deficiency of rainfall in these districts has been supplemented by irrigation from the perennial canals, however. To judge the extent of the area watered by canals consider that in 1868-69 about 1.37 million acres were irrigated while in 1918-19 and 1929-30 (including Indian States) about 9 and 12½ million acres were irrigated, respectively.

### **Rotation, Cultivation, Sowing, and Harvesting**

Wheat in Punjab is almost always sown on land that has been left fallow for several months after a previous crop of cotton, sugar cane,



wheat, gram (*Cicer arietinum*), or a millet. In the hills, however, owing to the smallness of holdings it is sown after maize (*Zea mays*) and rice, without any fallow. Generally eight to ten plowings on land dependent upon rains and five to six on irrigated lands are given. Only a small portion of wheat land is manured directly and then with ordinary farmyard manure alone.

Sowing begins late in September or early in October and continues until the end of December or even January, by far the greater portion of the area being sown between October 15 and November 15. Though sown in the fall, Punjab wheats can be spoken of as spring wheats, since they are rarely subjected to severe winter frosts. Generally four irrigations of a total amount of 10 to 12 inches are given canal-irrigated tracts, including the one given for sowing of the crop. When wells are the source of irrigation water the crop receives two or three irrigations more since the individual applications are lighter.

Harvesting begins at the end of March and the threshing is finished by the middle of June before the monsoon breaks.

### Varieties

Three species of wheats are grown in the Punjab: *Triticum sphaerococcum*, *T. durum*, and *T. vulgare*. Of the first group seven unit-species belonging to seven different varieties have been separated. These were found in dry districts of the southwest and are no longer cultivated. Four unit-species of the durum group belonging to four different botanical varieties have been separated. Only one of these, viz., type No. 1, belonging to the Malanopus variety, is economically important. Since the durumms need plenty of moisture and rich soil, their cultivation is restricted to only two or three districts of the Punjab where there is enough moisture. Durum wheat is used chiefly in the manufacture of semolina, which itself is used in making *halwa*, a kind of pudding. Durum is not exported. The third group, *T. vulgare*, is by far the most important, for it occupies most of the wheat acreage of the Punjab. About 65 unit species belonging to 20 different botanical varieties have been separated from the local mixtures. Of the types isolated, about two-thirds are beardless and one-third bearded, but as a class the former does not yield as much as the latter.

### Improvement of Wheat in the Punjab and the Purpose of this Investigation

In 1905 a beginning was made towards organizing an agricultural department on modern lines in the Punjab. At first 25 types of wheat were separated from the local wheat mixtures. Later surveys showed that local wheat crops were everywhere a mixture of types, of which

the most common were Types 11 (var. *erythroleucon*), 13 (var. *ferugineum*), 14 and 15 (*erythrospermum*), 16 (*graecum*), 17 (*Delfi*), 24 and 25 (*albidum*).

Economic improvement of wheat by the Department of Agriculture was started by distributing wheat type No. 9 (var. *pseudo-Hostianum*). It gave good yields on the Department's farms, but did not do well on farmers' land and was replaced by type No. 11, referred to above. This type was decidedly better than the local mixture and on the average gave  $1\frac{1}{2}$  to 2 *maunds* (1 *maund* =  $82\frac{2}{3}$  lbs.) of grain more per acre. Official distribution of seed of type No. 11 started in 1913 and in 1924-25 it had reached 930,000 acres. Thereafter its cultivation has been declining in favor of another type called 8A belonging to var. *Turicum*, which was first discovered as a single plant in a sheaf in one of the districts of the Punjab. This is a hard, amber-grained bearded wheat with reddish velvety chaff. Comparative varietal tests extending over a number of years conclusively showed that 8A yields  $1\frac{1}{2}$  to 2 *maunds* of grain per acre more than type No. 11 and more straw, which is a valuable fodder for cattle feeding in the Punjab. It was officially distributed to the farmers for sowing in 1919, and by the end of June, 1936, the area seeded to it was 3,161,700 acres. With the exception of Marquis, perhaps no other single product of wheat breeding anywhere has had so great a success as 8A. Numerous introductions of other wheat varieties have been made from Europe, America, Australia, Canada, etc. but not a single one has ultimately succeeded.

Although 8A still continues to be the premier wheat of the province, later observations showed that it had a few drawbacks:

- (1) In the Punjab, maize and sugar cane are always sown on land which has previously been heavily manured with farmyard manure. When wheat follows maize or sugar cane it generally lodges. This was the case with 8A. Land of this level of productivity is not very extensive, however.

- (2) Sometimes the farmer is compelled to seed his wheat as late as December or even January. This may be occasioned either by late rains in the *barani* area or by irrigation of the cotton crop, which is in the fruiting stage at that time, in preference to watering land for preparation of the seedbed for wheat sowing. Under such late-sown conditions it is desirable that the wheat be able to ripen in time; otherwise it is likely to be caught by the hot winds in early April, when the grain is still in the dough or hardening stage. This results in pinching of the grain and consequent diminution in yield. Being midseason in maturity, *i.e.* neither early nor late under the Punjab conditions, 8A takes full advantage of the normal Punjab wheat season.

Experience has shown, however, that this wheat when planted late is exposed to injury by hot winds and sometimes actually suffers when the hot winds set in earlier.

With a view to effecting improvement in some of the defects in 8A mentioned above, later researches have yielded three new wheats. These may be called the special-purpose wheats. These are C518, C591, and 9D. The first two are crossbred varieties and the last-named is a pure-line selection and belongs to the variety *pseudo-Hostianum*.

**C518.**—This is a fully bearded but comparatively short-awned amber-grained wheat with white, velvety chaff and grayish black awns. Ears are dense and erect. The straw is comparatively short and very stiff. It resists lodging to a very great extent, exhibiting this fault only on very rich soils. It yields better than 8A on lands of higher level of production, the combined average increase of 80 comparisons in six years 1929–30 to 1934–35 being  $3\frac{3}{8}$  maunds of grain per acre more than 8A. When good land and an adequate supply of water are available, C518 gives very good yields. The highest yield per acre with an authentic record to date was 65 bushels (Singh, 1933). This was a calculated acre yield, however, from a  $\frac{1}{2}$  acre plot.

**C591.**—This is fully bearded, amber-grained wheat with white, velvety chaff and grayish awns. This also resists lodging, though not as much as C518 but better than 8A. On good lands it yields better than 8A.

**9D.**—This wheat is a fully bearded wheat with white awns, white velvety chaff, and amber plump grains. It is recommended for sowing under the late-sown and *barani* conditions.

**Chapati-making properties of these wheats.**—As mentioned elsewhere most of the wheat produced in the Punjab is consumed in the form of chapatis (unleavened pan-baked cakes) prepared from whole-wheat meal. Therefore, in addition to high-yielding capacity and good milling and baking properties according to standards of European and other western countries, it is necessary, in order that it may meet with the approval of the farmers and general body of the consumers, that a new variety for distribution should possess satisfactory chapati-making qualities. Numerous tests in this direction have shown that all these wheats have good chapati-making properties. C591 is a superb wheat in this direction and gives perhaps the most tasty and excellent product. Second in the list comes 8A though C518 and 9D are more or less like it.

**Area under improved wheats in the Punjab.**—The total area sown to the improved wheats according to R. D. Singh <sup>2</sup> (personal communica-

<sup>2</sup> Cerealist, Punjab Agricultural College, Lyallpur, India.

tion to the author) up to 1936 has been estimated to be 4,661,700 acres, including, of course, 3,161,700 acres under 8A alone. This is about 50% of the total area of 9 to 10 million acres commonly sown to wheat in the Punjab.

*Progress of wheat hybridizing work.*—In order to evolve still better wheat having all-round good qualities, an extensive program of cross-breeding work is under way at the Botanical Farm, Lyallpur. During the six years beginning from 1929–30, 57 new crosses were made. These yielded about a hundred thousand (98,510) plants in the  $F_2$  generation. Each of these plants was studied singly and the selection of the desirable ones made from year to year. Twenty-three stable wheat-type samples out of 31 shown in Table III are selections made from some of the above cross-bred material. These are selected on the basis of their disease resistance and high-yielding capacities. No final decision can be made, however, so long as their quality is not examined. The purpose of the present investigations, therefore, is to test their milling, baking, and other physico-chemical qualities according to the fermented-bread-making methods. These can then be correlated with their chapati-making values and high-yielding powers. Any one of these proving satisfactory in all these respects can be finally selected for releasing to farmers for sowing.

### Material

A list of wheat types dealt with in the investigation is given in Table III. These wheats were raised on the Botanical Farm of the Punjab Agricultural Research Institute, Lyallpur, in 1935–36. Most of them were grown under irrigation. Some, however, were sown under *barani* conditions to test their suitability for such environment, because, as earlier pointed out, about 45% of the total wheat area in the Punjab every year is under *barani*. The irrigated plots were given four waterings and the *barani* only one and that merely to moisten the land for preparation of the seedbed for sowing and none thereafter. Rainfall during the year 1935–36, *i.e.*, from the first of July, 1935, to June, 30, 1936, was only 12.46 inches, which is 1.09 inches below the average of the 1926–35 decennial period. Of this precipitation, 5.33 inches occurred in the fallow period (monsoon season) and 2.6 in the growing period (winter rain), which was rather dry and moderately cold.

These types can be discussed under two different headings: (1) Serial numbers 1 to 8 are the duplicate samples of the four types, which are on the approved list of the Department of Agriculture and extensively grown by the farmers. (2) Numbers 9 to 31, with dupli-

TABLE III  
DESCRIPTION OF THE WHEAT SAMPLES USED IN THESE TESTS

Serial No.	Type No.	Parentage	Conditions of growth	Remarks
1	8A	Varieties on the approved list of the Department	Irrigated	Nos. 1-8 duplicated samples of types that are on the approved list of the Department of Agriculture, Punjab, and are being grown by farmers.
2	8A		"	
3	9D		<i>Barani</i>	
4	9D		"	
5	C518	H.F. × 9C	Irrigated	Nos. 9 to 31 are new cross-bred wheats under preliminary trials. Some of them are duplicate samples.
6	C518		"	
7	C591		"	
8	C591		"	
9	C205		"	
10	C206		"	
11	C207		"	
12	C207		<i>Barani</i>	
13	C208	C518 × H.F.	Irrigated	H.F. = Hard Federation. In writing parentage, the female parent is shown towards the right and the male parent towards the left.
14	C209		"	
15	C210		"	
16	C212		"	
17	C215	C516 × C591	<i>Barani</i>	C = Cross, meaning a hybrid. Irrigated means that the crop was irrigated by artificial means of irrigation, canal irrigation in this case.
18	C201	H.F. × 9D	Irrigated	
19	C201	"	<i>Barani</i>	
20	C202	"	Irrigated	
21	C202	"	<i>Barani</i>	<i>Barani</i> means dependent on rains. In Lyallpur, where these wheats were raised, the land was irrigated only once for seedbed preparation and no irrigation was given thereafter.
22	C203	"	Irrigated	
23	C204	"	"	
24	C224	"	<i>Barani</i>	
25	C225	"	"	
26	C227	"	"	
27	C227	"	"	
28	C228	"	"	
29	C228	"	"	
30	C229	"	Irrigated	
31	C229	"	<i>Barani</i>	

cate samples in some cases, are all recent hybrids, selected from much cross-bred material. These are still in the preliminary stage of trials.

Numbers 9 to 14 have Hard Federation and 9C as their parents. The former is a selection obtained from Australia, said to lodge less and to possess good milling and baking qualities. 9C, however, is an indigenous selection. This too possesses good milling and baking qualities. Nos. 15 and 16 are selected from the progeny of C518 and Hard Federation. No. 17, *i.e.* C215, is a composite cross, having C516 as one of its parents. C516 wheat, while establishing its unquestionable superiority to all other wheats against which it was tested under conditions of higher level of productivity, happened to be of very poor baking quality and had to be discarded. Efforts have been made to improve its quality, however, by combination with a better quality wheat, *i.e.* C591. Nos. 18 to 31 are all selections from the progeny of a cross between Hard Federation and 9D. The latter does not possess

acceptable milling qualities, probably because it has a low gluten content. Moreover, it has poor standing power. With a view to improving on these poor qualities, it has been crossed with Hard Federation.

Table VI gives the 1000-kernel weights of the samples. Each figure is an average value of two determinations; the average of the 21 samples comes to 41.4 g., with a range from 33.8 to 48.1. This shows the heaviness and plumpness of the kernels and less variation in the kernel weight.

This exceeds the average value for all India wheats (Table V) by

TABLE IV  
KERNEL WEIGHTS OF WHEATS GROWN IN MINNESOTA, 1911-1913<sup>1</sup>

Year	Spring wheat		Winter wheat	
	Number samples	Average 1000-kernel weight	Number samples	Average 1000-kernel weight
1911	97	g. 22.7	—	g. —
1912	106	25.2	47	27.6
1913	82	28.0	29	28.0

<sup>1</sup> Data from C. H. Bailey (1914).

TABLE V  
CERTAIN PHYSICAL AND CHEMICAL CHARACTERISTICS OF THE WHEATS GROWN IN INDIA  
(From the Government of India's Publication "Report on the Marketing of Wheat in India," 1937)

Province or state	Weight per bushel		Weight of 1000-kernels		Moisture		Crude protein		Crude dry gluten	
	Av.	Max.	Av.	Max.	Av.	Max.	Av.	Max.	Av.	Max.
Punjab—General Colonies	Lbs. 61.5	Lbs. 69.0	g. 32.3	g. 43.0	% 9.83	% 13.19	% 8.78	% 11.12	% 7.76	% 12.13
East and Southeast	61.4	64.7	31.6	40.9	9.99	12.84	8.64	11.17	7.19	9.31
East and Southwest	61.5	65.5	34.0	42.4	9.05	10.73	8.61	9.80	8.56	9.81
North	62.2	64.8	32.7	38.7	9.89	11.78	8.17	10.94	8.30	10.12
United Provinces:										
West	61.3	65.7	32.2	45.2	10.22	12.57	8.71	10.15 <sup>1</sup>	7.31	11.85
Central	62.7	66.2	32.8	44.4 <sup>2</sup>	10.72	12.67	9.59	10.37	8.45	10.23
East	62.7	64.8	32.5	41.0	11.23	—	13.57 <sup>3</sup>	—	7.02	—
U. P. Vulgare	61.2	66.3	36.8	45.6	10.44	11.66	9.24	11.12	8.30	10.35
Bombay Vulgare	62.6	64.5	37.7	44.7	10.90	12.35	10.17	13.05	11.35	14.73
Sind	60.8	65.5	31.7	39.9 <sup>4</sup>	9.67	10.53	9.97	12.60	9.59	13.55
Bihar and Orissa	62.4	64.5	30.6	38.6	9.78	11.40	9.42	12.77	8.45	13.25
N.W.F.P.	60.6	61.7	33.5	48.0	9.64	9.86	9.40	10.20	8.11	9.60 <sup>5</sup>
Bengal	61.4	65.7	30.6	38.6	9.26	11.35	10.79	13.51	9.47	11.49
Madras	60.6	63.3	35.7	45.6	—	—	—	—	—	—
Indian States:										
Rajputana	62.2	65.7	39.2	46.6 <sup>6</sup>	8.90	9.22	10.48	14.65	9.26	11.88
Central India:										
Vulgare	63.2	65.3	38.5	46.2	8.86	9.55	9.63	9.83	8.45	10.32
Hyderabad	60.3	65.3	39.2	47.8	10.81	11.53	10.31	13.97	10.73	16.90
Kashmir	60.8	63.9	33.9	41.8	10.84	11.88	8.63	9.58	6.98	8.84

<sup>1</sup> The protein content of a sample of Cawnpore 13 was 12.31%.

<sup>2</sup> One sample of Cawnpore 13 weighed 50.06 g. and a durum sample 50.20 g.

<sup>3</sup> Only one instance.

<sup>4</sup> The durum samples weighed 46.55 and 48.25 g.

<sup>5</sup> Only three samples analyzed.

<sup>6</sup> Five durum samples weighed 58.25, 57.05, 53.75, 53.20, and 51.50 g.

TABLE VI  
TEST WEIGHTS AND FLOUR YIELDS OF PUNJAB WHEATS

Number	Type	Irrig. or <i>barani</i>	Test weight per bushel of scoured wheat		1000- kernel weight of scoured wheat	Mot- tled grains	Yield of straight flour	Ratio between flour yield and bushel weight
			Win- chester	Im- perial				
1	C591	Irrig.	<i>Lbs.</i> 64.7	<i>Lbs.</i> 66.8	<i>g.</i> 47.3	% 6.6	% 80.0	1.23
2	C518	"	65.0	67.1	36.9	22.0	79.4	1.22
3	C518	"	65.0	67.1	34.1	21.3	78.9	1.21
4	C591	"	64.0	66.0	47.8	11.3	77.6	1.21
5	8A	"	63.5	65.5	33.8	1.3	77.1	1.21
6	8A	"	63.0	65.0	39.5	24.6	75.9	1.20
7	9D	<i>Barani</i>	64.6	66.7	38.2	56.3	68.5	1.06
8	9D	"	64.0	66.0	37.8	52.3	67.9	1.06
HARD FEDERATION × 9D								
9	C206	Irrig.	64.0	66.0	43.5	0.7	76.3	1.17
10	C208	"	64.0	66.0	45.1	0.7	75.5	1.18
11	C209	"	63.0	65.0	37.4	0.7	72.7	1.15
12	C205	"	65.6	67.7	43.1	1.0	72.3	1.10
13	C207	"	64.0	66.0	41.0	0.7	70.8	1.10
14	C207	<i>Barani</i>	65.0	67.1	41.2	0.7	69.7	1.07
C518 × HARD FEDERATION								
15	C210	Irrig.	63.0	65.0	48.1	8.0	75.5	1.19
16	C212	"	62.3	64.3	36.6	7.6	68.4	1.09
C516 × C591								
17	C215	<i>Barani</i>	64.5	66.6	39.4	4.6	77.4	1.20
HARD FEDERATION × 9D								
18	C225	Irrig.	64.0	66.0	40.9	11.6	80.0	1.25
19	C202	<i>Barani</i>	64.0	66.0	46.0	15.6	77.8	1.21
20	C229	"	64.0	66.0	40.9	4.6	75.8	1.18
21	C227	"	63.5	65.5	37.1	7.3	75.1	1.19
22	C204	Irrig.	63.3	65.3	47.1	3.0	75.5	1.19
23	C224	"	63.5	65.0	34.8	7.6	74.8	1.18
24	C203	"	63.5	65.5	45.7	24.0	74.1	1.17
25	C229	"	64.0	66.0	39.5	2.0	73.7	1.15
26	C201	<i>Barani</i>	63.6	65.6	47.0	12.6	71.9	1.13
27	C202	Irrig.	63.0	65.0	42.3	1.3	71.1	1.12
28	C228	<i>Barani</i>	64.0	66.0	44.1	14.0	70.9	1.10
29	C201	Irrig.	63.7	65.7	44.0	2.0	69.1	1.08
30	C228	<i>Barani</i>	64.5	66.6	46.2	6.3	69.0	1.07
31	C227	"	63.5	65.5	38.5	6.3	68.7	1.08

7.22 grams. The average for all Indian wheats is 34.18 g. and for the Punjab wheats 32.65 g., however.

Bailey (1914) reported the average 1000-kernel weights for some of the spring and winter wheats grown in Minnesota for the crop years 1912 and 1913. These values are given in Table IV for purpose of comparison.

### Test Weight per Bushel

Test weight per bushel is an approximate measure of the plumpness of the wheat kernels. It also gives an indication of potential flour yield, since, other things being equal, plump grains usually yield more flour than shriveled grains of low weight per bushel. The test weights of the scoured wheat samples are shown in Table VI, each value recorded being an average of three determinations. It will be seen that the average of 31 samples is 63.9 lbs., with a range from 62.3 to 65.6 lbs. per Winchester bushel. Again in Table V, the average test weight of all the wheats reported is in no case less than 60.0 lbs. per bushel. The maximum value of 69.0 lbs. per bushel is reported in the instance of wheats from the canal colonies of the Punjab.

### Relation between Test Weight and Flour Yield

Bailey (1924) summarized the results of two seasons' work at the Minnesota Experimental Flour Mill showing the correlation between weight per bushel and flour yield. Results by Bailey (1925) are given in the fifth table in his bulletin and indicated that flour yield generally followed weight per bushel.

Mangels and Sanderson (1925a) determined the correlation between test weights per bushel and yield of straight-run flour for seven crop years of hard red spring wheat. They found a high positive correlation and in all cases the coefficient of correlation was much higher than the probable error. The high degree of correlation is remarkable in that the data presented cover very different types of crop seasons.

A similar correlation between the test weight per bushel and flour yield was computed by the Harris formula in the instance of the data for the Punjab wheats recorded in Table VI and  $r$  was  $+0.219$ . This means that the correlation though positive was too small to be significant. Considering the whole range of possible bushel weights for these wheats, it is seen that they are assembled at the heavy end of the range of bushel weights of wheat. The grains of all samples are almost of the same uniform plumpness and therefore there is not much variation. When the range of material studied is narrow as in the range of bushel weights used here, the variation in the flour yield must be exceedingly small if the value of  $r$  is to be large enough to be sig-



nificant, and the error in determining flour yield may thus exceed the anticipated differences.

In the study made by Mangels and Sanderson, the range in test weight per bushel for the different crop years was 22.5 lbs. while the range in flour yield was 26 lbs. In this case, however, the variation is only 3.3 lbs. per bushel.

*Experimental milling.*—Each sample of 2,000 grams of cleaned wheat, plus the added water for conditioning, was milled in an experimental flour mill. This mill consists of four stands of Allis-Chalmers rolls and one double stand of Wolf rolls. The break rolls have differentials of  $2\frac{1}{2}:1$ , the smooth rolls  $1\frac{1}{2}:1$ . Each sample was subjected to five breaks and eight reductions with bolting after every grinding operation. The temperature of the mill room was under thermostatic control and a relative humidity of 85%–90% was maintained by a Bahnson humidifier.

*Flour yield.*—In flour yields, Indian wheats stand high among the wheats of the world. This is supported by the figures for flour yield given by Coleman *et al.* (1930), which show that among the wheat-producing countries of the world, India stands first in yield of flour from hard red winter and soft red winter grades and second in the instance of hard red spring and white wheat grades. The flour yield ranges between 71.5% and 74.2%. Fisher and Jones (1937) point out that with careful preparation before milling a good sound sample of Karachi wheat will actually give the highest percentage of flour of all the commercial classes, this condition depending upon adequate mellowing of the wheat during conditioning, which is not easy to attain.

When we compare the flour yields of 8A, C591, and C518, now constituting by far the major portion of annual acreage under the improved wheats in the Punjab, with the flour yields of some of the world wheats as given in the analytical data of world wheats by Geddes (1937), we find that these compare very favorably.

The average flour yield (calculated on total product, not on dry wheat) of the 31 samples studied is 73.9%, which is a rather high yield of flour when the relatively simple equipment used in the experimental milling is considered. With similar equipment Bailey produced 70.2% and 69.3% of straight-grade flour from Minnesota spring and winter wheats respectively.

Eight samples of wheats C591, C518, 8A, and 9D, which are the improved wheats extensively grown at present by the Punjab farmers, yielded 75.6%. Thus C591 and C518 give a 79%–80% yield, while 8A yields 75.9% to 77% and 9D almost 68%. It is probable, however, that the 80% yield of C591 was a little too high because of the low

yield of bran, which may have been the result of under-conditioning. The second sample of C591 (serial No. 4, Table III) was therefore milled by adding 1% more water, *i.e.* 18% moisture content and the same for C518 and 8A (serial Nos. 2 and 6).

Among the new hybrids cross No. 225 gave the highest flour yield, 80%. Next was cross No. 202 (*barani*), giving 77.8%. A few others also gave a good flour yield.

### Crude Protein in Punjab Wheats and Flours

The crude protein contents of wheat and flour of the samples, calculated to 13.5% moisture basis, are recorded in Table VII.

The average crude protein content in wheat of the 31 samples was 10.36%. In the first group of eight samples of C518, C591, 8A, and 9D the average crude protein was 9.3%, of which 9D had the least amount. There was a difference of 1.7% between two samples of 8A. This may have been due to the physical consistency of the two samples—one having 1.3% of mottled grains while in the second sample mottling was as high as 24.6% (Table VI).

Percentage of crude protein in the hybrid samples of wheat having Hard Federation or 9D as one of their parents was the highest, ranging from 10.6% to 12.2% with an average of 11.45%. This may have been the result of a good combination of their two parent wheats, both of which are reputed to possess good milling and baking qualities.

In the third group, *i.e.* hybrids resulting from a cross between Hard Federation and 9D, the level of crude protein was decidedly higher than in the first group. The average was 9.71% with a range of from 9.6% to 10.8%, excepting one unusual instance which was as high as 12.43%. In other words, a combination of 9D and Hard Federation has yielded hybrids which have from 1.5% to 2.7% more protein than 9D itself, which has only 8.1%.

Attention should be called to cross No. 215, a hybrid of C516 and C591. Its protein content was 9.7%, the same as that of C591. As mentioned elsewhere, C516 has very poor baking quality, and the above combination does not appear to have yielded results of any economic importance.

Reference is again made to Table V regarding the distribution of protein in wheat in different parts of India. The average ranges between 8% and 10.8% with a single instance of 13.57%. In the Punjab, the average protein content of the wheat previously reported was 8% to 9% with a maximum of 11.17%. The level of protein in the samples analyzed by us was higher, however, which suggests that a decided improvement is being and has been made on the existing stock,

TABLE VII  
CRUDE PROTEIN AND CRUDE GLUTEN CONTENTS OF PUNJAB WHEATS

Type No.	Moisture content		Crude protein (N×5.7), 13.5% moisture basis		Crude gluten		
	Wheat	Flour	Wheat	Flour	Wet	Dry	Ratio
	%	%	%	%	%	%	
8A	11.2	12.1	10.65	9.57	24.7	9.4	2.62 : 1
8A	11.4	12.9	9.10	8.32	20.2	7.8	2.59 : 1
9D(B) <sup>1</sup>	11.3	13.1	8.06	7.35	17.9	6.8	2.63 : 1
9D(B)	11.5	13.7	8.16	7.35	18.6	6.9	2.69 : 1
C518	10.8	12.4	9.68	9.35	23.5	8.8	2.67 : 1
C518	10.8	13.3	9.63	9.10	24.7	9.0	2.74 : 1
C591	11.3	13.1	9.67	8.57	24.0	9.0	2.66 : 1
C591	11.3	13.8	9.70	8.81	24.6	8.8	2.81 : 1
HARD FEDERATION × 9D							
C205	10.8	12.4	10.55	9.68	28.6	10.3	2.78 : 1
C206	10.8	12.8	12.19	10.83	31.3	11.2	2.79 : 1
C207	10.9	12.5	12.10	11.36	29.2	11.3	2.58 : 1
C207(B)	10.9	13.6	11.21	10.84	29.7	10.7	2.78 : 1
C208	11.7	12.8	10.89	10.10	29.0	10.6	2.74 : 1
C209	11.0	13.2	11.80	11.06	29.3	10.0	2.93 : 1
C518 × HARD FEDERATION							
C210	11.4	12.8	10.58	9.70	27.2	9.5	2.86 : 1
C212	10.9	13.3	11.19	10.81	26.6	10.1	2.63 : 1
C516 × C591							
C215	11.3	12.6	9.71	8.94	25.1	8.7	2.89 : 1
HARD FEDERATION × 9D							
C201	11.1	14.5	10.70	9.95	25.1	9.6	2.61 : 1
C201(B)	11.1	13.3	10.41	9.62	24.9	9.6	2.59 : 1
C202	11.2	13.5	12.43	11.34	28.6	11.0	2.60 : 1
C202(B)	11.1	13.7	10.49	10.00	24.0	9.4	2.55 : 1
C203	11.5	12.9	10.17	9.01	24.4	9.0	2.71 : 1
C204	11.6	13.1	10.23	9.43	26.2	9.0	2.91 : 1
C224	10.9	12.4	9.80	8.93	23.5	8.7	2.70 : 1
C225	11.0	12.9	10.14	9.38	26.0	9.2	2.83 : 1
C227(B)	11.0	12.7	10.75	10.01	28.2	10.1	2.79 : 1
C227(B)	11.0	12.9	10.75	10.12	27.8	10.0	2.78 : 1
C228(B)	11.2	12.4	10.25	9.52	28.2	9.7	2.91 : 1
C228(B)	11.4	12.9	10.23	9.89	29.3	10.1	2.90 : 1
C229	11.2	12.2	9.60	8.94	30.4	9.3	3.27 : 1
C229(B)	11.8	12.4	10.38	9.85	28.9	9.9	2.92 : 1

<sup>1</sup> (B) denotes *barani*.

or that the crop season or the area in which these samples were grown resulted in a higher than normal protein content.

When we compare these wheats with hard bread wheats grown in other parts of the world, we find that as regards percentage of crude protein these wheats, with an average of 10.36%, are in the medium-to-low range. It is of the same order of magnitude as the softer types of

wheats reported in the tri-state (Ohio, Indiana, and Michigan) surveys by Bayfield (1933) which ranged as follows:

YEAR	AVERAGE CRUDE PROTEIN %
1931	10.67
1932	9.80
1933	11.26
1934	11.64
1935	9.88
1936	9.64

### Crude Protein in Wheat and Physical Texture of the Wheat Kernel

Grain texture, as determined by the percentage of vitreous, mottled, and soft grains in a wheat sample, bears a certain relation to protein content and consequently to baking strength. The physical texture of the grain of the samples is recorded in Table VI, and the protein content in Table VII. Samples of 9D have the highest percentage of mottled grains and the lowest of protein content, while C205-208 all have mottled grains less than 1%, and the percentage of crude protein is proportionately higher, perhaps highest. Coleman, Fellows, and Dixon (1925) have also shown that dark, hard, and vitreous kernels have more protein than the soft and chalky ones.

This relation between kernel texture and protein in wheat has been statistically examined by Mangels and Sanderson (1925b), Hayes, Immer, and Bailey (1929), Singh (1935), and Aamodt and Torrie (1935). The conclusion arrived at by these workers is that there exists a significant positive correlation between the vitreousness of the kernels and the protein in wheat.

A similar correlation was worked out in this case, with the difference that in this case the percentage of mottled grains was correlated with the percentage of protein in wheat. The correlation has been found to be significant but negative, indicating that mottling of grain is inversely proportional to the protein content ( $r = -.66 \pm .0675$ ).

### Crude Gluten

Crude gluten in the experimentally milled flours was determined by the A. A. C. C. method (1935). The percentage of, and the ratio between, wet and dry gluten (dried to constant weight in an air oven at 97° to 99° C.) is shown in Table VII. The results corroborate the statement, made earlier, that the percentage of dried crude gluten is approximately equivalent to the percentage of crude protein in flour.

### Diastatic Activity

The determination of diastatic activity of the flour samples shown in Table VIII was made by the Blish and Sandstedt (1933) method as

TABLE VIII  
 DIASTATIC ACTIVITY  
 (Maltose produced in 10 g. flour in one hour)

Type	Maltose	Type	Maltose	Type	Maltose
	mg.		mg.		mg.
C591	289	Hard Federation × 9C		Hard Federation × 9D	
C518	286	C208	276	C204	291
8A	283	C209	268	C229	259
9D	274	C207	237	C225(B) <sup>1</sup>	258
				C228	257
C591	271	C206	230	C224	256
C518	269	C205	221	C203	254
9D	266			C201	253
8A	258	C518 × Hard Federation		C202	241
		C210	272	C202(B)	235
				C202	235
		C516 × C591		C229(B)	231
		C215	238		

<sup>1</sup> (B) denotes *barani*.

described in A. A. C. Cereal Laboratory Methods (1935). It will be seen that the diastatic activity of these samples seems to have been fairly high, ranging between 221 and 291 with an average of 256.7, with the exception of two or three cases. The range of variation was not great, however. The diastatic activity of the C591, C518, 8A and 9D wheat group was high; average of these eight samples was 274.5. Next comes the group of hybrids, having Hard Federation and 9D as parents, with an average of 251.7, while among the progeny of Hard Federation and 9C it was 246.7.

### Chapati-Making Quality

The bulk of the Punjab wheat is consumed in the form of *chapati* (unleavened pan-baked cakes). Generally the quality of *chapati* can be judged by two factors: (1) its softness and flexibility, which may perhaps be due to the quantity and quality of protein in the flour, and (2) its sweet and flavory taste which may be due to the amount of sugar in the flour plus the hydrolysis of starch into sugar by the diastatic enzymes as a result of addition of water to the flour to make dough. Therefore a wheat in order to be of good *chapati*-baking value should have, no doubt, a fairly high diastatic activity.

Of the wheats listed in the upper group (Table IX), C591 and 8A make very good *chapaties* of excellent flavor, C591 perhaps the better, and C518 almost as good as 8A. Good *chapaties* cannot be made from 9D, however. This may perhaps be due to less protein in the flour. Of the 31 samples analyzed, 9D has been shown to possess the least amount of protein both in the wheat and the flour.

Another point in the testing of the quality of *chapati* is its keeping quality. The common practice is to bake them fresh for every meal. A soft silky *chapati* on keeping, say overnight, will not have its softness impaired or lessened to a very great extent. This may be due to the water-holding capacity of protein.

Sufficient water is added to the flour to make it into a fairly stiff dough. This dough consists of flour and water only. It is kneaded with the hand and allowed to stand for an hour or so, so that the moisture absorption is complete and the dough assumes a definite shape and consistency. It is kneaded again for a short time before baking.

The dough then is divided into small dough balls. Each dough ball is rolled and flattened and made into a *chapati*, which is then put on the hot plate. After a minute or two the side of the *chapati* in contact with the hot plate becomes stiff as a result of dehydration of protein and starch. It is then turned over so that the other side gets stiff likewise. It is then either turned over again on the hot plate or put on the glowing charcoal, where it puffs and becomes ready for use. It is usually greased with butter before eating and is always eaten when it is fairly warm.

#### Relation Between Diastatic Activity and the Protein Content of Flour

If we examine the diastatic activity and the protein content of these flours, we find that the averages of these two variables are as follows:

GROUP	AVERAGE OF	DIASTATIC ACTIVITY mg.	PROTEIN IN FLOUR %
Duplicate samples of C518, C591, 8A, and 9D	8 samples	274.5	8.55
Hybrid (H.F. $\times$ 9D)	11 samples	251.7	9.58
Hybrid (H.F. $\times$ 9C)	5 samples	246.4	10.64

The fact, as suggested by the above data, that the diastatic activity appears to have an inverse relation with the protein content of flour warranted statistical examination of the case. The coefficient of correlation between these two variables was, therefore, computed with the following result:  $r = -.422 \pm .1067$ .

This shows that as far as these data are concerned there was a small negative correlation with a large probable error, however.

While diastatic activity is not directly associated with, or due to the protein content of, wheat, it apparently occurs not uncommonly that certain factors of environment during the late growth period of the plant influence both variables concurrently. Thus arid or drought conditions tend to result in a high-protein wheat grain. The same conditions also seem to result in a high degree of biological maturation of the grain which implies a low enzymic activity in general. Thus the

diastatic activity tends toward a minimum when the protein content is approaching an abnormally high level.

A natural corollary of these conditions is a tendency towards dry, well-cured wheat under drought conditions, which implies a minimum of field-damaged and sprouted kernels. This also maintains a minimum of diastatic activity in the grain.

### Determination of the Gassing Power of Flour

In order to study the relation between the diastatic activity and gassing power, four flours of varying diastatic activity were selected. The method employed was the same as adopted by Bailey and Johnson (1924). In terms of volume of gas production and dough expansion, C591 gave the maximum values, C518 was second, C229 was third, and C201 was lowest, which is also the order of their diastatic activity. To what extent the diastatic activity is influenced by the environment in which wheat is grown can be judged by the fact that the wheat C229 when grown under rain-dependent (*barani*) conditions showed a diastatic activity of 254 and the same wheat under the irrigated condition gave only 176, a difference of 78 units. Similar observations were made by Mangels (1926) and Swanson (1935).

### Water Absorption

Water absorptions of the flour samples under investigation were determined by the farinograph except in some cases where this had to be done by hand kneading of 25 g. of flour, in duplicates, by the addition of necessary amounts of distilled water to make dough of a workable consistency. The amount of water used multiplied by four is recorded in the case of hand-kneading tests.

The water absorptions are given in Table IX. It is evident that these flours have a very high water absorption, the average of 31 samples being 72.6%. This is all the more remarkable in view of the average protein in the flour, which was only 9.60% and considerably lower than the bread wheats of United States, Canada, and Russia, etc. What this absorption may be due to cannot be explained at present. This is a problem requiring further elucidation.

Reference is made to the summary of milling and baking qualities of the world wheats, Coleman *et al.* (1930), page 218. It will be seen that Indian wheats belonging to the predominating classes of hard red winter and white stand very conspicuously at the top in water absorption. In this respect they compare still more favorably with the world wheats (Geddes, 1937). Fisher and Jones (1937), page 95, also found the water absorption of Karachi wheat to be relatively very high  $\frac{72.6}{9.60}\%$  a correspondingly high bread yield.

TABLE IX  
WATER ABSORPTIONS AND BAKING RESULTS

Serial No.	Type	Water absorption			Two min. mixing, 1 hr. fermentation		One min. mixing, 1½ hrs. fermentation		Change in bread properties from 1st to 2nd baking method
		By farinograph	Used in baking	Difference	Loaf vol.	Baking score	Loaf vol.	Baking score	
		%	%	%	cc.		cc.		
1	8A	72.0	72.0	+0.0	510	56.0	515	53.5	0
2	8A	70.6	73.0	+2.4	440	45.0	475	53.5	+
3	9D	70.6	75.0	+4.4	485	—	452	51.2	—
4	9D	68.0	75.0	+7.0	525	60.5	480	54.0	—
5	C518	76.8	79.0	+2.2	455	46.5	495	54.5	+
6	C518	72.0	74.5	+2.5	465	50.5	500	51.0	+
7	C591	77.3	81.0	+3.7	485	—	403	36.3	—
8	C591	68.6	72.0	+3.4	500	55.0	500	50.0	0
C516 × C591									
9	C215	66.8	70.0	+3.2	515	63.5	455	52.5	—
HARD FEDERATION × 9C									
10	C205	68.6	70.0	+1.4	530	59.0	525	60.5	0
11	C206	69.3	71.0	+1.7	490	52.0	570	67.0	++
12	C207	68.3	72.0	+3.7	485	50.5	613	74.3	++
13	C207B	67.0	68.5	+1.5	605	69.5	615	68.5	+
14	C208	75.0*	75.0	+0.0	535	63.5	538	58.8	0
15	C209	76.0	77.0	+1.0	580	72.0	425	44.5	--
C518 × HARD FEDERATION									
16	C210	78.0*	77.0	-1.0	550	67.0	518	58.8	—
17	C212	66.6	69.0	+2.4	515	55.5	500	54.0	—
HARD FEDERATION × 9D									
18	C201	64.4*	65.0	+0.6	515	50.5	508	53.8	0
19	C201B	71.3	73.0	+1.7	510	53.0	485	49.5	—
20	C202	70.1	70.1	+0.0	440	46.0	465	43.5	+
21	C202B	68.4	71.0	+2.6	505	53.5	470	47.0	—
22	C203	71.0*	71.5	+0.5	440	45.0	445	45.5	0
23	C204	75.2	76.0	+0.8	500	53.0	493	55.3	0
24	C224	69.6	74.0	+4.4	455	50.5	525	60.5	++
25	C225	70.5	74.0	+3.5	575	69.5	525	62.0	—
26	C227	67.8	69.0	+1.2	500	58.0	545	63.5	+
27	C227	68.0*	71.0	+3.0	550	67.0	535	62.5	—
28	C228	73.3	75.0	+1.7	535	61.5	508	57.8	—
29	C228	70.0*	72.0	+2.0	580	70.0	500	55.0	—
30	C229	67.2*	67.5	+0.3	465	54.5	500	58.0	+
31	C229	71.3	73.0	+1.7	485	57.5	480	58.0	0

\* Determined by hand kneading.



### Protein Content and Water-absorbing Capacity of Flour

It is generally thought that high-protein flours have higher water-absorbing capacity and *vice versa*, although this is not invariably the case, as considerable variation in absorption may be encountered in wheat flours of the same protein content but milled from different lots.

Mangels (1928) worked out coefficients of correlation for four crop years. He found positive correlations for three years between  $+0.2$  and  $+0.3$  and a negative correlation in one year. He felt that coefficients of this magnitude do not indicate a high degree of correlation between protein content and water absorption.

In order to ascertain whether any relationship exists between the protein content and water absorption in the instance of the flours involved in these studies, the coefficient of correlation of these two variables was computed and found to be  $r = +0.6747 \pm 0.066$ . This shows that so far as these data are concerned there is a high positive correlation.

### Baking Tests

For experimental baking test the standard test formula of the American Association of Cereal Chemists was followed except that the sugar was doubled. The formula used was as follows:

Flour 100 g. (100%) at 15% moisture  
Yeast 3 g. (3%)  
Salt 1 g. (1%)  
Sugar 5.0 g. (5%)

In the light of information yielded by the farinograph, that the dough formation of these flours took less time and the range of minimum dough mobility was rather narrow, it was thought advisable not to subject them to a long high-speed mixing or long fermentation. The low range of protein in these flours was another factor in favor of short fermentation. Accordingly a test baking, with two minutes of mixing and one hour of fermentation, was conducted. The loaf-volume figure in each case is a measurement of a single loaf, and accordingly too much reliance cannot be placed on these results. Nevertheless they give some indication of baking quality when they are compared with the results of the second baking.

It had been observed in this laboratory that the amount of water added to certain types of flour for bread making is generally 1% to 2% less than determined by the farinograph when titrated to 500 Brabender units. The same thing was done in this case, but it was found that the dough was quite stiff. Water was then added equal to that found by the farinograph and the dough still felt too stiff. Finally 1% to 2%

more water than the farinograph determination was given. The second baking was done by the addition of 1% to 4% more water; in some cases even more. Low loaf volumes with sample Nos. 10, 11, 12, 24, and 26 in Table IX very probably are due to insufficient water for optimum dough consistency.

In a second series of baking tests the mixing time was reduced from two minutes to one minute and the fermentation period was increased from 1 hour to 1½ hours. The results are shown in Table IX. Each figure is an average of two loaf-volume measurements. This is almost of the order of American soft winter wheats as reported by Bayfield (1933).

There is, however, one factor to be taken into consideration and that is that this average of 502 cc. is the result of great variations in the loaf volume due, perhaps, to the different genetical composition of the hybrids. When the selection is made amongst those which have higher loaf volume as well as other desirable characteristics much more favorable results are apparent.

If we compare the loaf volumes of these flours yielded by two different baking methods (Table IX), we find that there were some which gave increased loaf volume when mixing time was decreased to 1 minute and fermentation time increased to 1½ hours. There were others which did not respond to the changed treatment, and again there were some which gave decreased loaf volumes. Where the change from the first to second treatment was negligible it is indicated by zero. In the remaining cases the magnitude of such a change is expressed by the number of (+) or (-) signs for the positive and negative change respectively. On the whole, crosses 205, 206, 207 (Hard Federation × 9C) and crosses 224, 225 and 227 (Hard Federation × 9D) have given all around good results.

### Viscosities of Flour-in-Water Suspensions

The standard A. A. C. C. method detailed in Cereal Laboratory Methods (1935) was employed. A MacMichael viscosimeter with a No. 30 wire disk bob and speed of 12 rpm. was used. All the determinations were made at room temperature (80°F.). The suspensions were prepared without previous removal of electrolytes, and they were acidulated with lactic acid. The results reported in Table X are averages of duplicate determinations of 24 selected samples. These results show two sets of conditions: First, where the maximum viscosity occurs at the point when the first cubic centimeter of lactic acid is added. The second addition of 2 cc. decreased the viscosity a great deal. The additions of subsequent third and fourth 2-cc. portions

TABLE X  
VISCOSITIES OF FLOUR-IN-WATER SUSPENSIONS

Type	Viscosity, degrees MacMichael					Increase or decrease (—) in viscosity from added acid (°MacM.)				Type of vis- cosity
	Acid used, cc.					Acid used, cc.				
	0	1	3	5	7	1	3	5	7	
C518	11.15	44.5	10.5	12.5	17.5	33.0	—34.0	2.0	5.0	A+1
C518	9.0	39.5	8.5	12.0	16.5	30.5	—31.0	3.5	4.5	A+1
C591	11.0	50.5	13.5	11.0	13.0	39.5	—37.0	—1.5	2.0	A+2
C591	7.5	41.5	8.0	9.5	13.0	34.0	—33.5	1.5	3.5	A+1
8A	9.0	25.0	10.0	15.5	21.0	16.0	—15.0	5.5	5.5	A
8A	10.5	25.5	11.0	10.0	13.0	15.0	—14.5	—1.0	3.0	A
9D	11.0	21.5	17.5	26.0	32.0	10.5	—4.0	8.5	6.0	B
9D	9.0	16.5	13.0	22.5	27.5	7.5	—3.5	9.5	5.5	B
C518 × HARD FEDERATION										
C210	9.0	29.0	11.0	20.0	26.5	20.0	—18.0	9.0	6.5	A
C212	7.5	20.0	11.5	20.5	27.0	12.5	—8.5	9.0	6.5	B
C516 × C591										
C215	8.5	19.0	17.0	27.0	32.0	10.5	—2.0	10.0	5.0	B
HARD FEDERATION × 9C										
C205	6.5	10.0	25.5	38.0	42.0	4.0	15.5	12.8	4.0	B+1
C206	8.0	12.0	30.0	47.0	52.0	4.0	18.0	17.0	5.0	B+2
C207	5.0	8.5	38.5	46.0	50.0	3.5	30.0	7.5	4.0	B+2
C209	10.0	33.0	10.5	14.5	19.5	23.0	—22.5	4.0	5.0	A
HARD FEDERATION × 9D										
C201	5.0	8.0	20.5	26.5	29.5	3.0	12.0	6.0	3.0	B
C201	8.0	14.5	20.5	33.5	39.6	6.5	6.0	13.0	6.0	B
C202	7.5	13.0	15.5	25.5	31.5	5.5	2.5	9.5	6.5	B
C203	7.5	15.5	12.5	23.5	28.0	8.0	—3.0	11.5	4.5	B
C204	11.0	18.6	30.5	49.5	52.5	7.0	12.5	19.0	3.0	B+2
C224	9.0	10.0	31.5	44.0	51.5	1.5	21.0	12.5	7.5	B+2
C225	9.0	12.0	31.0	43.5	51.0	3.0	19.0	12.5	8.0	B+2
C227	11.0	13.0	26.5	40.0	42.0	2.0	13.5	13.5	2.0	B+1
C228	8.0	12.5	30.0	43.5	49.0	4.5	17.5	13.5	5.5	B+2
Amer. wheat flour	8.0	10.5	41.5	63.5	71.0	2.5	31.5	21.5	8.0	B+2

tended to increase the viscosity but not to an appreciable extent. In the second case, however, the increase in viscosity was approximately proportional to the successive increments of the acid and the maximum viscosity occurred on the addition of 7 cc. of acid.

On the basis of the above distinctions these wheat samples have been classified according to the type of viscosity exhibited by each. The first case, where the maximum viscosity occurred with the addition of 1 cc. of acid, has been designated by *A*, *A+1*, and *A+2*, depending upon the extent of increase, *A* representing the lowest maximum and *A+2* the highest maximum.

The other condition (with 7 cc. of acid) is represented by *B*, *B+1*, and *B+2*, *B* representing the lowest maximum and *B+2* the highest maximum. The arbitrary limits have been fixed as follows:

<i>A</i>	Less than 35°	MacMichael by the addition of 1 cc. acid
<i>A+1</i>	Between 35°-45°	MacMichael by the addition of 1 cc. acid
<i>A+2</i>	More than 45°	MacMichael by the addition of 1 cc. acid
<i>B</i>	Less than 35°	MacMichael by the addition of 7 cc. acid
<i>B+1</i>	Between 35°-45°	MacMichael by the addition of 7 cc. acid
<i>B+2</i>	More than 45°	MacMichael by the addition of 7 cc. acid

According to the two divisions mentioned above into which these wheats have resolved themselves as regards viscosity behavior two things become apparent:

(1) C518, C591, 8A and 9D exhibit viscosities of the *A* type. The change in viscosity is very great in the first two, less in 8A, and least in 9D. C215, whose parents both happened to be the indigenous wheat hybrid selections, behaved in like manner.

(2) In the case of hybrids resulting from a combination of the local wheat selections with Hard Federation, a foreign bread wheat, the viscosity behavior in the majority of cases is shown to be analogous to the bread wheats of the United States or Canada, for example, though the maximum viscosity is less than these, perhaps because of less crude protein in wheat and flour.

So far as the literature is concerned no case has been recorded where the maximum viscosity occurred by the addition of first 1 cc. of lactic acid and then the following addition of 2 cc. acid decreased the viscosity. The reason for this is not apparent and constitutes a problem requiring further research. This behavior might be suspected to be a function of the hydrogen-ion concentration of the preparations, pH of the flour-in-water suspensions having been shown previously to influence the viscosity. When pH was determined the problem was not clarified, save that in C518 and C591 the maximum viscosity occurred at pH 4.5 while in the other case the maximum viscosity occurred at pH 2.8 to 3.0. The viscosity measurements in °MacM and

the relative pH of the four typical flours with different increments of acid are shown in Table XI.

TABLE XI  
VISCOSITIES AND pH DETERMINATIONS

Treatment 1N lactic acid	American wheat		C206		C518		C591	
	pH	Viscosity	pH	Viscosity	pH	Viscosity	pH	Viscosity
cc.		°MacM.		°MacM.		°MacM.		°MacM.
—	5.78	8.0	5.78	8.0	5.98	11.5	5.93	7.5
1	4.41	10.5	4.38	12.0	4.56	44.5	4.51	41.5
3	3.58	41.5	3.58	30.0	3.77	10.5	3.80	8.0
5	3.18	63.0	3.07	47.0	3.38	12.5	3.42	9.5
7	2.98	71.0	2.82	52.0	2.77	17.5	2.81	13.0

C518 and C591, viscosity A type; American wheat and C206, viscosity B type.

### Carotenoid Pigments

The method described by Ferrari and Bailey (1929) was used in the extraction and estimation of the carotenoid pigments and the results for the different flours examined are recorded in Table XII as the average of two observations. The range of carotenoid pigment content was from 2.50 to 1.42 parts per million. The flours milled from wheats on the approved list, 8A, 9D, C518, and C591, have a carotenoid content of 2.05 or above, while there is some variation among the new hybrids.

TABLE XII  
CAROTENOID PIGMENT CONTENT OF DIFFERENT WHEAT SAMPLES

Serial No.	Type or cross No.	Parentage	Carotenoids
			ppm.
1	8A	—	2.50
2	C207	H.F. × 9C	2.33
3	C591	—	2.27
4	9D	—	2.23
5	C212	C518 × H.F.	2.15
6	C518	—	2.05
7	C215	C516 × C591	2.00
8	C205	H.F. × 9C	1.90
9	C206	H.F. × 9C	1.80
10	C225	H.F. × 9D	1.80
11	C202	H.F. × 9D	1.77
12	C202B	H.F. × 9D	1.75
13	C208	H.F. × 9C	1.75
14	C225	H.F. × 9D	1.68
15	C228	H.F. × 9D	1.62
16	C201	H.F. × 9D	1.55
17	C209	H.F. × 9C	1.55
18	C210	C518 × H.F.	1.54
19	C203	H.F. × 9D	1.47
20	C204	H.F. × 9D	1.45
21	C229	H.F. × 9D	1.42

With the exception of C207 and C212 the rest have a carotenoid content less than 2.0 and as low as 1.42 ppm. It is possible that this may be due to less pigment in Hard Federation, which is one of the parents of these hybrids.

If we compare the range of the carotenoid pigment content of these wheats with wheats of the world, we find that they compare favorably. Worzella and Cutler (1935) have presented (page 710) the results of carotenoid contents of 29 soft and semi-hard American wheat varieties for the crop year 1932-33 and 1933-34, which ranged from 1.70 to 3.80 in 1933, and 1.80 to 3.80 in 1934 with an average of 2.37 and 2.41 ppm., respectively.

Whiteside, Binnington, and Geddes (1933) have given carotene values of gasoline extracts of straight-grade flours of Marquis and Garnet wheats grown at 14 different places in Western Canada in 1930. For Marquis the carotene values ranged between 1.44 and 2.69 with an average of 1.97 and for Garnet between 1.79 and 3.86 with an average of 2.45 ppm.

### Farinograph Study

The farinograph curves of some of the flour samples are shown in Figure 4. It will be observed that in case of C591, C518, 8A, and 9D

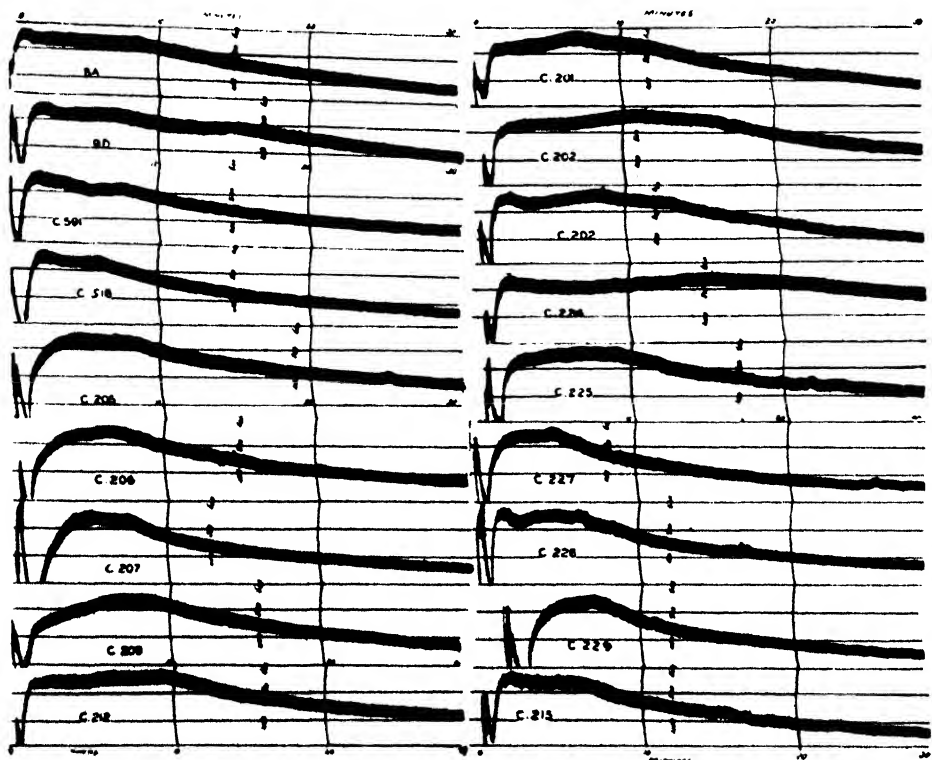


Fig. 4. Farinograph curves of Punjab flours.

the time required for dough development was rather short. This is a characteristic of many typical soft wheats. The dough mixing stabilities of C591 and C518 were lower than were those of 8A and 9D. Although the protein content in 9D was least of all, the nature of the curve suggests a good quality of protein. C215 (C516  $\times$  C591) behaved almost exactly like C591, neither of which appeared to possess a good quality of protein.

Some of the hybrids resulting from the crossing of Hard Federation  $\times$  9C and Hard Federation  $\times$  9D possess relatively superior colloidal dough properties so far as can be determined by farinograph curves. An all-round improvement seems to have been accomplished by hybridizing yet the dough-mixing stability is much less than for bread wheats of the United States and Canada. Cross 224 (Hard Federation  $\times$  9D) showed a distinct improvement over 9D in both quality and quantity of protein, while C205, C206, C207, C224, and C225 gave good baking results as well.

### Quantitative Determination of the Degree of Dough Softening

Softening can be well illustrated by a reference to a farinogram in Figure 5. The maximum plasticity (minimum mobility) of the dough

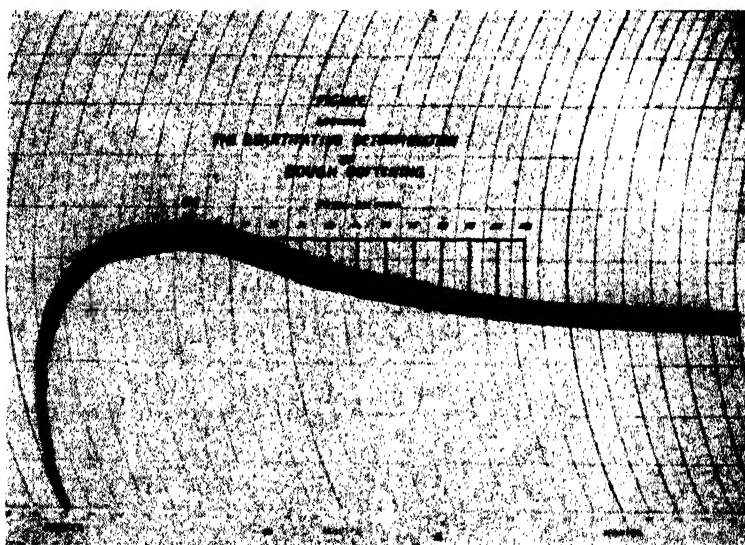


Fig. 5. Quantitative measurement of dough softening.

appears to be attained at the end of the sixth minute and begins to decline after 6.5 minutes. Just where the curve begins to fall, a point is marked and every minute thereafter up to twelve minutes. A horizontal line is projected from the first point and the distance between the

points marked on the curve and the parallel line is measured and expressed in terms of farinograph units. In this case the value of the first distance is 5, of the second 20, and so on up to the twelfth minute. All these values are then summated. The sum (895 units in this case) expresses the amount of the softening of the dough. The abrupt or gentle fall in the curve indicates the degrees of softening. These values are comparative only and give an indication of the degree of resistance to mixing which a dough offers to the mechanical abuse to which it is subjected. The summated values of the extent of dough softening of the flour samples are given in Table XIII.

TABLE XIII  
RESULTS OF FARINOGRAPHIC STUDY WITH WHEAT FLOUR SAMPLES

Type	Max. dough development		Departure from minimum mobility (softening) at end of successive minutes (1-12)												Summation of 12 "softening" values
			1	2	3	4	5	6	7	8	9	10	11	12	
	Units	min.	Units												
8A	550	1.25	15	15	20	22	22	25	24	30	38	50	60	65	386
9D	540	2.5	20	20	20	20	20	28	40	46	50	50	50	50	414
C518	550	2.5	18	20	20	23	40	60	75	85	95	105	110	120	771
C591	550	2.0	18	22	35	45	40	40	50	60	70	90	100	100	670
C516 × C591															
C215	550	2.25	10	15	10	15	20	50	65	80	95	95	110	110	675
HARD FEDERATION × 9C															
C205	530	5.0	5	6	7	18	30	45	60	65	75	80	90	95	576
C206	540	6.5	5	20	35	50	60	70	80	95	110	115	125	130	895
C207	545	4.5	6	0	5	25	45	60	75	95	105	115	120	130	775
C207(B)	540	5.0	5	20	40	50	68	80	90	100	110	120	123	130	936
C518 × HARD FEDERATION															
C212	550	3.0	5	5	0	0	0	0	0	0	10	20	50	55	145
HARD FEDERATION × 9D															
C201	520	2.0	0	0	0	0	0	0	0	0	0	0	10	25	35
C202	530	3.0	0	0	0	0	0	0	0	0	0	0	0	0	0
C202(B)	550	2.5	20	15	10	0	0	0	0	5	10	15	30	40	145
C224	530	2.25	10	20	20	25	25	20	15	10	10	5	0	0	160
C225	555	7.0	0	4	15	25	45	60	70	75	80	90	100	105	669
C227	540	4.0	0	10	30	55	70	80	90	100	110	120	125	135	925
C228	550	2.5	20	5	0	5	20	35	50	70	80	95	105	110	595
C229	540	6.0	25	45	70	80	95	110	120	130	135	140	145	150	1245



### Brabender's Single-Figure Evaluation of Farinograms

Recently Brabender (1937) has formulated a method with which the farinogram of a flour dough is evaluated by a single-figure score. This consists in combining the three variables, *viz.* the time in minutes of maximum dough development, degree of dough softening in units, and the single-figure score, in a three dimensional chart. First the difference between maximum consistency and consistency after 12 minutes of mixing is noted. This difference is matched on the chart against the time required to reach maximum consistency. The relative single-figure score is then directly read on the chart. A high score is presumed to imply superior quality. The results of evaluating the curves by the single-figure-score method are given in Table XIV.

TABLE XIV  
SINGLE-FIGURE SCORES

Type	Max. dough development		Fall after 12 min.	Difference	Single-figure score
	<i>Min.</i>	<i>Units</i>	<i>Units</i>	<i>Units</i>	
8A	1.25	550	485	65	47.0
9D	2.5	540	490	50	53.5
C518	2.5	550	430	120	41.3
C591	2.0	550	440	100	42.8
C516 × C591					
C215	2.5	550	440	110	42.0
HARD FEDERATION × 9C					
C205	5.0	530	435	95	55.5
C206	6.5	540	410	130	60.0
C207	4.5	545	415	130	50.0
C207(B) <sup>1</sup>	5.0	540	410	130	52.5
C518 × HARD FEDERATION					
C212	3.0	550	495	55	53.5
HARD FEDERATION × 9D					
C201	2.0	520	495	25	58.5
C202	3.5	530	530	0	64.0
C202(B)	2.5	550	510	40	55.4
C224	2.25	530	530	0	62.0
C225	7.0	555	450	105	63.0
C227	4.0	540	405	135	47.0
C228	2.5	550	440	110	43.0
C229	6.0	540	390	150	56.0

<sup>1</sup> (B) denotes *barani*.

### Dough Softening and Single-Figure Scoring of Farinograms

In Table XV are the summated values giving the extent of dough softening as a result of the breaking down of dough structure in 12

minutes after the minimum dough mobility was recorded. In the right-hand column are the results of single-figure evaluations of the farinograms. These results do not exhibit any definite relationship. The coefficient of correlation between these two variables was computed and the value of  $r = -.035 \pm .157$ , which is an insignificant coefficient.

TABLE XV  
DATA REGARDING DOUGH SOFTENING AND SINGLE-FIGURE  
SCORING OF THE FARINOGRAMS

Serial No.	Type	Summated values <sup>1</sup>	Type	Single-figure score <sup>2</sup>
<i>Units</i>				
1	C202	0	C202	64.0
2	C201	35	C225	63.0
3	C202(B) <sup>3</sup>	145	C224	62.3
4	C212	145	C206	60.0
5	C224	160	C201	58.5
6	8A	186	C229	56.0
7	9D	414	C205	55.5
8	C205	576	C202(B)	55.4
9	C228	595	9D	53.5
10	C225	669	C212	53.5
11	C591	670	C207(B)	52.5
12	C215	675	C207	50.0
13	C518	771	8A	47.0
14	C207	775	C227	47.0
15	C206	895	C228	43.0
16	C227	925	C591	52.8
17	C207(B)	936	C215	42.0
18	C229	1245	C518	41.3

<sup>1</sup> Arranged in ascending order.

<sup>2</sup> Arranged in descending order.

<sup>3</sup> (B) denotes *barani*.

### Summary

Results of a biochemical and technological study of some Punjab wheat varieties grown on the Botanical Farm of the Punjab Agricultural Research Institute, Lyallpur, India, in 1935-36 are reported. Thirty-one samples were used in this study, of which 8 samples (duplicates) 8A, 9D, C518, and C591, are improved wheat types, on the approved list of the Punjab Department of Agriculture and extensively grown by the farmers. Out of a total acreage of 9 to 10 million acres annually cropped to wheat, these four types occupied as much as 50%, and 8A alone occupied 3,161,700 acres. The remaining 23 samples are all recent hybrids selected from the cross-bred material. These are still in the experimental stage. The purpose of this investigation was to determine their milling and baking qualities according to the fermented-bread-making standards and methods.

The average 1000-kernel weight of these 31 samples was 41.4 g. with a range of between 33.8 and 48.1 g., which indicates plump, heavy

kernels and moderate variation in the kernel weights. The average of these samples exceeds the average value for all India wheats by 7.22 grams.

The average test weight per Winchester bushel of these samples was 63.9 lbs. with a range from 62.3 to 65.6 lbs.

Indian wheats as a rule are classed as dry wheats, since the period during which they are harvested and threshed is a very hot and dry one. Coleman *et al.* (1930) give moisture data between 9% and 10% for some of the Punjab wheats examined. The moisture contents of samples reported here were a little higher, ranging between 10.8% and 11.8%. The small variation may be due to the fact that wheats tend to absorb moisture in storage during the monsoon (rainy) period.

In flour yields, Indian wheats stand high among the wheats of the world. This is supported by the data of Coleman *et al.* (1930) and Geddes (1937). The average flour yield of the 31 samples tested was 73.9% and that of the samples of C518, C591, 9D and 8A was 75.6%.

Among the new hybrids C225 gave the highest flour yield, 80%, and the next in order was C202 (*barani*), yielding 77.8% flour.

The coefficient of correlation computed between the bushel weight and flour yield was +.219. The correlation, though positive, is too small to be significant, which may have been due to the uniformly high bushel weights of these wheats. So far as crude protein in wheat was concerned, these wheats fell within medium-to-low range with an average of 10.36%. The group containing 8A, 9D, C518, and C591 averaged 9.3%. In the group of hybrids with Hard Federation  $\times$  9C as parents the average crude protein was 11.45%, ranging from 10.6% to 12.2%. This may have been due to the combination of wheats both of which are reputed to possess good milling and baking qualities.

The average crude protein of all India wheats ranges between 8% and 10.8%, that previously reported for the Punjab being between 8% and 9%. The level in these samples was, however, higher, which shows an improvement on the existing stock. The coefficient of correlation was computed between crude protein in wheat and the percentage of mottled grains in each sample, with  $r = -.66 \pm .06775$ . Crude gluten generally equals the crude protein in flour.

Diastatic activity ranged between 221 and 291, with an average of 256.7. With the exception of two or three cases the range of variation was not very large. The average of the group of approved wheat varieties (8A, 9D, C518 and C591) was 274.5. Compared with data for world wheats reported by Geddes, these wheats are high in diastatic activity.

The bulk of the Punjab wheat is consumed in the form of *chapaties* (unleavened pan-baked cakes). Therefore the method of *chapati* baking and testing is described.

The water absorption of flours milled from these wheats averaged 72.6%. This is very high indeed in view of the low average protein content.

A high positive correlation between the water absorption of flour and protein content value was observed,  $r = +.6747 \pm .066$ . Mangels found a low positive correlation, between 0.2 and 0.3, which did not indicate a high degree of correlation between these two variables in the samples tested by him.

The average loaf volume was 502 cc. with a maximum of 615 cc., which is of the order of American soft winter wheats. There is one factor to be taken into consideration, however, that this average was the result of a great variation in the loaf volume due perhaps to the different genetical make-up of the hybrids. On the whole crosses 205, 206, 207 (Hard Federation  $\times$  9C) and crosses 226, 225, and 227 (Hard Federation  $\times$  9D) gave good baking results.

The results of viscosity determinations show that among these wheats there existed two sets of conditions: the first, or *A* type, where the maximum viscosity occurred at the point when the first cubic centimeter of lactic acid was added, the second addition (2 cc. acid) decreased the viscosity substantially, while a third and fourth addition of 2 cc. acid each tended to raise the viscosity, though not to a substantial extent. In the second case, or *B* type, the increase in viscosity was fairly regular with successive increments of the acid and the maximum viscosity occurred on the addition of 7 cc. of acid. Indigenous selections almost all show *A* type, while hybrids resulting from a cross where one of the parents is Hard Federation exhibit the *B* type of viscosity. The literature does not record instances where the maximum viscosity occurred with the addition of the first cubic centimeter of lactic acid and the following addition of 2 cc. of acid decreased the viscosity.

The pH of each system was determined, and in the *A* type the maximum viscosity occurred at pH 4.5 while in the *B* type the maximum viscosity occurred at pH 2.8 to 3.0.

Carotenoid pigment of the flours ranged from 2.50 to 1.42 parts per million. Samples of 8A, 9D, C518, and C591 had a carotenoid content above 2.05 ppm., and there was some variation among the new hybrids.

Farinograms show that the dough development times of these flours with few exceptions were short. The range of dough stability was also comparatively narrow. Some of the hybrids of Hard Federation  $\times$  9C

and Hard Federation  $\times$  9D showed better dough properties, however, so far as was evident from their farinograms. Evaluation of the farinograms was made by two methods, (1) the summation of the decrease in consistency at intervals of one minute for 12 successive minutes after the maximum consistency or minimum mobility was reached, and (2) Brabender's new method of single-figure scoring of farinograms. No relation between these two evaluations appeared in these instances ( $r = -.035 \pm .157$ ).

### Acknowledgments

Grateful acknowledgment is made of the counsel given by Dr. R. A. Gortner during the course of certain portions of this study. Our sincere thanks are extended to H. R. Steward, Director of Agriculture of the Punjab, who generously provided the wheat samples, and to R. D. Singh for access to his data, and for his stimulating suggestions.

### Literature Cited

- American Association of Cereal Chemists  
1935 Cereal laboratory methods.
- Aamodt, O. S., and Torrie, J. H.  
1935 Studies on the inheritance of and the relation between kernel texture and protein content in several spring wheat crosses. *Canadian J. Res.* C13: 202-219.
- Bailey, C. H.  
1914 Minnesota wheat investigations, Series III. Composition and quality of spring and winter wheats. Crops of 1912-13. *Minn. Agr. Exp. Sta. Bul.* 143.  
1924 Report of operations of State Testing Mill for crop season of 1922. *Minn. State Dept. Agri. Bul.* 34.  
1925 The chemistry of wheat flour. Chemical Catalog Company, New York.  
— and Johnson, A.  
1924 Carbon dioxide diffusion ratio of wheat flour dough as a measure of fermentation period. *Cereal Chem.* 1: 293-304.
- Bayfield, E. G.  
1933 The quality of soft winter wheats as influenced by phosphate and other soil treatments. *Proc. World Grain Exhibition and Conference, Regina, Canada* 2: 447-458.
- Blish, M. J., Chairman Baking Test Committee  
1928 Proposed reporting system for standard baking test. *Cereal Chem.* 5: 289-296.  
— and Sandstedt, R. M.  
1933 An improved method for the estimation of flour diastatic value. *Cereal Chem.* 9: 189-202.
- Brabender, C. W.  
1937 Beitrag zur Weizenstandardisierung. Ein Vorschlag für den Weltweizenstandard. *Nordisk Cerealkjemikerforenings og Nordisk Cerealistforbunds*, pp. 152-163. Oslo, 1937.
- Coleman, D. A., Fellows, H. C., and Dixon, H. D.  
1925 A study of methods for making protein tests on wheat. *Cereal Chem.* 2: 132-164.
- Coleman, D. A., *et al.*  
1930 Milling and baking qualities of world wheats. *U. S. Dept. Agr. Tech. Bul.* No. 197.
- Ferrari, C. G., and Bailey, C. H.  
1929 The determination of carotin in flour. *Cereal Chem.* 6: 347-371.
- Fisher, E. A., and Jones, C. R.  
1937 The wheats of commerce. *Tech. Educ. Series Pamphlet No. 10, Research Assn. Brit. Flour Millers.*

- Geddes, W. F.  
1937 The development of new wheat varieties of high milling and baking quality. *Le Congrès Internat. Tech. et Chim. des Industries Agr.* 2: 148-161. Scheveningue, Hollande.
- Government of India  
1937 Report on the marketing of wheat in India.
- Hayes, H. K., Immer, F. R., and Bailey, C. H.  
1929 Correlation studies with diverse strains of spring and winter wheats, with particular reference to inheritance of quality. *Cereal Chem.* 6: 85-96.
- Johnson, A. H., and Bailey, C. H.  
1925 Gluten of flour and gas retention of wheat flour doughs. *Cereal Chem.* 2: 95-106.
- Mangels, C. E.  
1926 Factors affecting the diastatic activity of wheat flour. *Cereal Chem.* 3: 316-322.  
1928 Relation of water absorbing capacity of flour to protein content, baking quality, and loaf weight. *Cereal Chem.* 5: 75-77.
- and Sanderson, T.  
1925a Correlation of test weight per bushel of hard spring wheat with flour yield and other factors of quality. *Cereal Chem.* 2: 365-369.  
1925b The correlation of the protein content of hard red spring wheat with physical characteristics and baking quality. *Cereal Chem.* 2: 107-112.
- Singh, R. D.  
1933 Wheat in the Punjab. *Proc. World Grain Exhibition and Conference, Regina, Canada*, 2: 22-28.  
1935 Summary report of consolidated results of completed experimental work carried out by the Cerealist, Punjab Agricultural College, Lyallpur, during the quinquennium 1930-31-1934-35. Unpublished.
- Swanson, C. O.  
1935 Some factors which affect the diastatic activity in wheat. *Cereal Chem.* 12: 89-107.
- Whiteside, A. G. O., Binnington, D. S., and Geddes, W. F.  
1933 The quantitative determination of carotenoid pigments in wheat and flour. *Proc. World Grain Exhibition and Conference, Regina, Canada* 2: 438-447.
- Worzella, W. W., and Cutler, G. H.  
1935 Carotenoid pigments in wheat with special reference to varieties and strains. *Cereal Chem.* 12: 708-713.

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## A STUDY OF THE EFFECTS OF PROTEOLYTIC ENZYMES AND $\text{KBrO}_3$ UPON THE VISCOSITY AND ALLIED PROPERTIES OF DISPERSIONS OF HARD RED SPRING WHEAT GLUTEN

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(Received for publication July 13, 1939)

The use of viscosity determinations in the study of the effects of various factors upon protein characteristics is quite familiar to cereal technologists. The underlying principles of these methods rest presumably upon the concentration, configuration, and size of the protein particles.

Vogel and Bailey (1927) found the viscosities of leached and acidulated suspensions of durum wheat flour to be definitely lower than

like preparations of *vulgare* wheat flour of the same concentration. Morgan (1924) cited the utility of the viscosity test when applied to flour-water suspensions in determining flour quality.

Gortner (1924) developed a method whereby the viscosity of flour-water suspensions could be used to evaluate the quality of glutenin. Sharp and Görtner (1924) determined the viscosity of a number of acidulated dough-water suspensions made from fermenting dough. Samples of dough were taken at different periods during fermentation and the effect of fermentation upon gluten properties was determined. The viscosity of the suspensions following removal of electrolytes was found to increase as fermentation progressed. Malt flour and extract greatly diminished this effect owing to their proteolytic action.

Sharp and Gortner (1923) applied viscosity measurements of flour-water suspensions to the investigation of the comparative imbibitional properties of the flour protein. An effort was also made to correlate the colloidal properties of the protein with flour strength. These workers postulated that glutenin was the protein mainly responsible for the marked imbibition of gluten. An inherent difference in the physico-chemical properties of gluteins from strong and weak flour was affirmed. These differences were found to be related to the colloidal state of the gluten proteins. Their report contains an excellent summary of literature dealing with viscosity measurements as applied to colloidal solutions. Blish and Sandstedt (1925) using more than 100 samples of Nebraska wheat flours studied the relationship between viscosity and baking quality. A relationship was found between actual viscosity and loaf volume. The order of this correlation was essentially the same as that between protein content and loaf volume. Actual viscosity and protein content were more highly correlated than other factors, and it was concluded that protein content forms as valuable a basis for the prediction of baking strength as the actual viscosity values.

Sharp (1926) investigated the plasticity of flour-water suspensions using a simple apparatus in which pressure from an ordinary hand-pressure pump was applied to force the material through a capillary. Various pressures were used. A particular flour-water suspension was found to be plastic when it contained 9% or more of flour by dry weight.

Johnson (1927) investigated the viscosity of flour-water suspensions at different extraction temperatures and various lengths of extraction period. The effect of degree of manipulation was also studied. The hydration value of flour particles was found to undergo a sharp change at 50° C. The rate of proteolytic activity of the flour suspension could be determined by viscosimetric techniques only in cases where

the degree of hydration and electrolyte content of the suspension were held constant when the viscosities of several suspensions digested for different periods of time were determined.

Johnson and Herrington (1928) found that the flour suspension which exhibited the highest viscosity contained approximately 50% of the total nitrogen. These workers also found (1928a) that certain proteins present in wheat flour caused a depression of the viscosity of acidulated flour suspensions.

Johnson, Herrington, and Scott (1929) measured proteolysis in digested flour-water suspensions using a method whereby the viscosity was determined after leaching and acidulating the suspension made from the residue following decantation of the supernatant liquid. The rate of decrease in viscosity was found to be greater at higher temperatures. The authors concluded that the formol titration method is probably the most convenient procedure for determining the proteo-clastic activity of flour, but no information was yielded regarding hydrolytic changes of the protein micelle.

Johnson and Green (1930) showed the effect of fermentation upon the viscosity of dough-water suspensions. The rate of decrease in viscosity depended upon the H-ion concentration during fermentation, while the presence of NaCl and buffer salts prevented the decrease in viscosity during fermentation periods as long as 10 hours. This decrease in the absence of added salts was irreversible. Johnson (1931) proved that the viscosities of flour-water suspensions extracted with solutions of the potassium and sodium halides were higher than those of similar suspensions extracted with distilled water. The decrease in viscosity was according to the lyotropic series of the anions. Glutenin was not removed by the extraction with halides.

Jong and Klaar (1930) studied the viscosity changes induced in gliadin sols by varying alcohol and H-ion concentration. The viscosity of both positive and negative gliadin sols was found to increase up to 48% of alcohol by weight. Higher alcohol concentrations caused a marked decrease in viscosity for both positive and negative sols. The maximum viscosity was supposedly caused by the formation of an alcohol hydrate layer around the particles. Higher alcohol concentrations would remove this layer. The question whether an emulsoid will flocculate, unmix, or remain in solution by discharging apparently depends upon the isoelectric hydration.

Denham, Scott Blair, and Watts (1927) studied the utility of the Ostwald type of viscometer for purposes of measuring the viscosities of flour suspensions up to 30% concentration. This instrument proved to be entirely satisfactory provided it was calibrated with solutions of known viscosity, and the results were expressed in absolute units. It



was found that the capillary dimensions might be raised within wide limits without affecting the accuracy of the results, provided the flow is laminar within the range of the instrument, and that the critical velocity was not exceeded. Scott Blair, Watts, and Denham (1927) judged that viscosity measurements of flour suspensions at different concentrations would be useful for determining flour strength. An empirical formula ( $\log \eta = k''c + d$ ) was found advantageous for plotting viscosity against concentration,  $k''$  and  $d$  being constants. Plotting these values was found to yield a true straight line in all instances.

Bayfield (1933) reported the conclusions reached by the subcommittee on the viscosity tests for soft-wheat flour. This test, employing the MacMichael viscometer, is widely used by soft-wheat milling laboratories and is generally considered to be of distinct value in connection with quality evaluation. By means of this test it appeared probable that relatively small differences due to variety of wheat, ash, and protein content might be detected. Later Bayfield (1935) postulated that protein quantity was readily measured by viscosity determinations. Quality of protein, however, proved to be more difficult to determine, although when several wheat varieties were compared marked differences were observed.

Rose and Cook (1935) found that the viscosity as determined on protein dispersions with the U-tube viscometer yielded valuable information regarding particle size, hydration and denaturation changes in these dispersions when they were prepared by different methods.

Landis and Frey (1938) described a method for measuring proteolytic activity through changes in the rate of gelation of gelatin sols following exposure to proteolytic agents. Harris (1938) published the conclusions derived from a study of the effects of various proteoclastic enzymes upon wheat-gluten "solubility" in sodium salicylate solution. It was shown that in the majority of experiments enzyme action markedly hastened the dispersion of the gluten, and this effect increased with an increase in concentration of enzyme. Inhibition of enzymic activity by  $\text{KBrO}_3$  was demonstrated, especially in the instance of papain and bromelin. Later this study was extended to investigations into the effects upon the relative distribution of the protein fraction removed from sodium salicylate gluten dispersions by additions of  $\text{MgSO}_4$ . Striking changes in the proportion of protein removed in the first fraction were evident when papain, malt, and taka-diastase were added to the dough mix from which the glutes were subsequently washed. This effect was not evident in the pepsin treatment. Balls and Hale (1938) applied a viscosity method with gelatin to the measurement of the proteolytic activity of wheat-flour proteinase prepared by them.

In view of the substantial amount of work which has been done with flour-water suspensions in an endeavor to relate viscosity values to flour quality, it was judged expedient to apply such methods to the elucidation of viscosity changes in connection with the action of proteolytic enzymes upon gluten washed from doughs treated with such enzymes. The Ostwald pipette is a convenient form of capillary instrument to use for this purpose. Relatively small quantities of liquid are required for a reading, the pipette and contents are easily held at a constant temperature, and readings covering a substantial range in viscosity may be made with one instrument. Mangels and Bailey (1933) and Mangels (1934, 1936) used the Ostwald pipette for purposes of studying the viscosities of a large number of starches prepared from hard red spring and durum wheats with satisfactory results.

### Experimental Material and Methods

A commercially milled hard red spring wheat flour was used in this investigation. The flour had been neither bleached nor diastated in the mill and contained 13.1% protein and 0.51% ash on a 13.5% moisture basis. The malt-phosphate-bromate loaf volume was 625 cc.

The glutens were washed from doughs mixed in the usual manner for baking, using a two-minute mix in the Hobart-Swanson mixer. The formula used included 1% salt, 3% yeast, 5% sucrose, and distilled water to firm consistency. The glutens were washed immediately after mixing, under a small standardized stream of 0.1% sodium phosphate which had a pH of 6.8. This procedure corresponds essentially to the technique formerly used by the senior author in work of this nature and already reported (Harris, 1938). The gluten was divided and placed in 10% sodium salicylate solution with thorough shaking. The concentration used in the first part of this work was 6 g. of wet crude gluten per 100 cc. of sodium salicylate solution. Shaking was continued at 5-minute intervals and aliquots taken for viscosity determinations at hourly intervals for the first two hours, then at two-hour intervals until the course of dispersion had been clearly established. Approximately 35 cc. of liquid were decanted into 50-cc. centrifuge tubes from this suspension following a settling period of five minutes from the last shaking to allow suspended shreds and particles of gluten to settle. These aliquots were then centrifuged and the density and viscosity determined. After these determinations the liquid as well as the residual material deposited in the centrifuge tubes was returned to the original dispersion flask. In this way no permanent change in the concentration of the liquid in relation to the undispersed material was caused by removal

of the aliquot. Since only 5 cc. of liquid were required for the measurement of viscosity, the bulk of the colloidal solution was returned when the density had been ascertained. By this technique, the total 35 cc. were separated from the actual mixture only during the centrifuging and the weighing of the specific gravity bottle.

The viscosity determinations were carried out with an Ostwald pipette. This pipette, and the dispersions preliminary to testing, were held at  $25 \pm 0.1^\circ \text{C}$ . in a constant-temperature bath fitted with heater, thermoelectric regulator, and stirring device. This apparatus

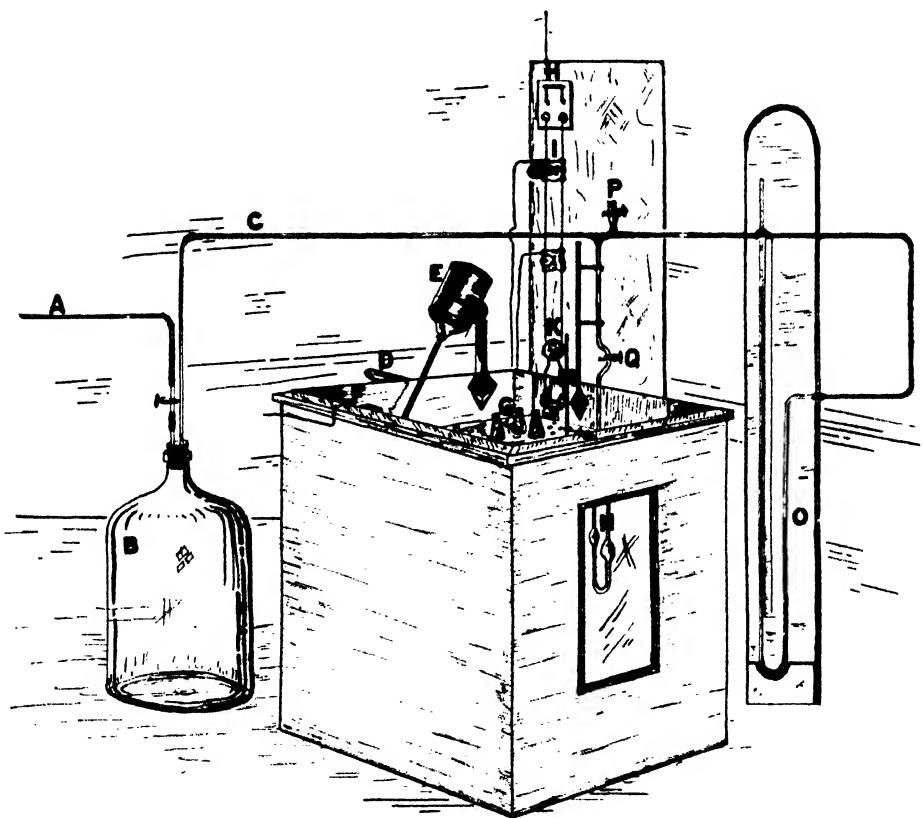


Fig. 1. Illustration of constant-temperature bath and accessory apparatus used in determining the viscosities and rates of flow.

is shown in Figure 1. The bath itself was constructed of wood and lined with sheet copper. Glass windows were installed at each end of the bath. Above the window at the left in the illustration a light and reflector (*D*) served to illuminate the Ostwald pipette (*N*) which could then be read through the window shown on the right in the figure. A copper shelf (*G*) served both as baffle plate and as a holder for flasks containing the various liquids and dispersions worked with, to insure that the contents were held at the temperature of the bath. The bath and contents were kept at a constant temperature by a

thermoregulator (*F*) which was placed in parallel with the pilot light (*I*), heater switch (*K*), and heater (*L*). A switch (*H*) led to the source of current. The water was kept in circulation by a stirrer (*E*). The difference between atmospheric pressure and a small partial vacuum was used as a driving head to force the liquid through the pipette rather than the application of direct pressure upon the liquid. It is assumed that the partial vacuum which served to draw the liquid through the capillary of the Ostwald pipette was equivalent to an applied pressure which would force the liquid through. To accomplish this purpose, a large carboy (*B*) was partially evacuated by a glass tube (*A*) which led to a water-suction pump. A glass tube (*C*) united the carboy to the mercury manometer (*O*) and to the pipette through a four-way glass tube and suitable connections. The degree of vacuum obtained was read from the manometer. A valve (*P*) served to release the vacuum in the system when the determination was finished. The three-way valve (*Q*) was used to connect the pipette to the partial vacuum and to the air.

A series of pressures was used on each liquid examined. The mode of procedure was to start at a low partial vacuum and raise the pressure by small increments between successive determinations. The pressure used varied from the weight of the liquid in the bulb of the pipette to the same weight increased by approximately 100 mm. of mercury. When measuring the increase in viscosity with time of dispersion, the pipette was disconnected from the glass tube and two-way valve, thus insuring free flow of liquid without extraneous resistance in the pipette capillary.

### Discussion

The effects of different papain concentrations in comparison with 0.004% and 0.016% increments of  $\text{KBrO}_3$  upon the viscosities of the gluten dispersions are shown in Table I. These determinations were

TABLE I  
EFFECT OF VARIOUS ADDITIONS OF PAPAIN AND POTASSIUM BROMATE TO DOUGHS  
UPON VISCOSITY CHANGES IN GLUTEN DISPERSIONS

Dough treatment	Viscosity (centipoises $\times 10^3$ )								$dc/dt^1$
	1st hr.	2nd hr.	4th hr.	6th hr.	8th hr.	10th hr.	12th hr.	Final	
Control	131.1	137.9	155.2	172.8	182.9	190.3	197.4	219.9	8.5
Papain 0.004%	132.0	139.2	161.1	184.7	195.3	199.1	203.3	203.9	9.6
Papain 0.004% + $\text{KBrO}_3$ 0.004%	128.6	135.7	158.9	179.2	193.2	201.8	209.5	192.4	8.5
Papain 0.004% + $\text{KBrO}_3$ 0.016%	127.8	131.5	149.9	168.5	186.2	197.2	206.5	197.6	7.0
Papain 0.008%	135.3	150.7	182.6	197.7	197.3	194.8	194.8	188.2	13.4
Papain 0.008% + $\text{KBrO}_3$ 0.004%	129.5	140.6	168.0	188.9	198.8	198.6	198.3	188.9	11.9
Papain 0.008% + $\text{KBrO}_3$ 0.016%	129.2	134.3	160.8	181.8	194.8	197.7	201.1	184.8	9.2
Papain 0.02%	168.7	170.1	169.8	170.7	170.6	169.0	169.0	166.6	41.4
Papain 0.02% + $\text{KBrO}_3$ 0.004%	162.1	171.7	167.1	167.0	167.0	167.0	167.0	161.3	34.8
Papain 0.02% + $\text{KBrO}_3$ 0.016%	152.6	174.6	169.4	168.5	168.5	168.5	168.1	163.5	25.3
Papain 0.03%	162.0	161.0	160.0	161.2	158.9	159.6	159.6	160.5	

<sup>1</sup> Calculated by use of interpolated values from Figure 2.

run over a period of 12 hours. The results are also shown graphically in Figure 2, where viscosity is plotted against time. Owing to the nature of the data, the results are more easily interpreted from the figure and the discussion will be largely confined to it, rather than the table. From the results obtained with papain alone it will be noted that the effect of the enzyme is markedly to increase the viscosity during the initial hours of dispersion. This increase becomes more

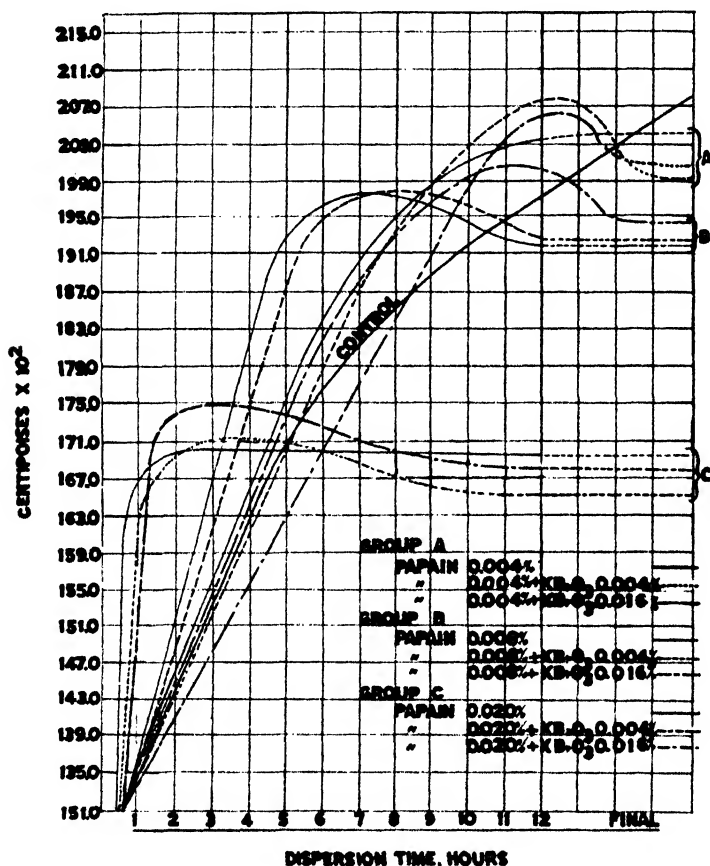


Fig. 2. Effects of papain and potassium bromate upon the increase of viscosity with time of dispersion in gluten dispersions.

marked at higher papain dosages. The effect reaches a maximum and tends, in some instances, to decrease slightly at the final hour, although this decrease is probably not significant. When KBrO<sub>3</sub> was also added to the doughs, in addition to papain, a lower rate of dispersion was at first evident in every instance. These results of bromate action upon papain are also shown in Table I under the column headed  $dc/dt$ , which represents rate of change with increase in time. It is apparent from these results that papain by itself greatly increases this value, while bromate in each instance lowers the value obtained in the unbromated

papain treatment. The fraction  $dc/dt$  represents the slope of the lines from the starting point until the time when the line begins to curve, showing the effect of other factors such as structure, configuration, and size of particle upon the viscosity, rather than increase in gluten concentration in the dispersion. This fraction was calculated with interpolated values taken from the figures. The equation representing the slope, or the rate of disintegration, is:

$$\frac{dc}{dt} = \frac{\eta a - \eta_0}{ta - to},$$

where:  $\eta a$  = viscosity at the point where the line begins to curve.

$\eta_0$  = viscosity at the zero hour—27.3.

$ta$  = time corresponding to  $\eta a$ .

$to$  = time corresponding to  $\eta_0$  or 0.

In the majority of cases, the final viscosity of the bromated dough-gluten dispersions fell below the unbromated dough-gluten value and would tend to indicate some effect upon the particle size or properties, corresponding to a slight activation of the papain. This is especially noticeable in the lowest 0.004% bromate treatment, but is not apparent in the intermediate 0.008% papain-treated dough gluten. This effect of  $KBrO_3$  upon viscosity of papain-treated doughs will be discussed in more detail a little later in this paper.

In Table II similar data obtained with three increments of pepsin and corresponding bromate dosages are shown, while Figure 3 presents

TABLE II

EFFECT OF VARIOUS ADDITIONS OF PEPSIN AND POTASSIUM BROMATE TO DOUGHS UPON VISCOSITY CHANGES IN GLUTEN DISPERSIONS

Dough treatment	Viscosity (centipoises $\times 10^3$ )								$dc/dt^1$
	1st hr.	2nd hr.	4th hr.	6th hr.	8th hr.	10th hr.	12th hr.	Final	
Control	131.1	137.8	158.1	189.1	216.5	224.3	231.0	233.4	9.8
Pepsin 0.1%	132.2	135.2	154.7	178.0	200.9	213.7	223.0	238.7	8.8
Pepsin 0.1% + $KBrO_3$ 0.004%	134.8	136.5	159.0	177.4	201.6	215.1	220.1	232.7	9.1
Pepsin 0.1% + $KBrO_3$ 0.016%	132.1	136.3	148.9	165.8	190.6	200.1	211.8	233.1	7.9
Pepsin 0.3%	139.2	158.8	195.2	210.4	218.1	219.6	219.3	225.6	16.9
Pepsin 0.3% + $KBrO_3$ 0.004%	149.9	179.5	206.3	215.1	216.5	220.9	220.9	210.4	21.9
Pepsin 0.3% + $KBrO_3$ 0.016%	140.7	159.5	189.8	207.1	215.5	222.7	222.7	220.9	14.9
Pepsin 0.5%	194.3	197.4	196.4	197.0	199.0	199.0	199.0	196.5	67.0
Pepsin 0.5% + $KBrO_3$ 0.004%	197.1	201.9	199.8	199.9	199.9	199.9	199.9	199.7	69.8
Pepsin 0.5% + $KBrO_3$ 0.016%	191.5	201.0	199.6	199.7	199.7	199.7	199.7	200.4	64.4

<sup>1</sup> Calculated by use of interpolated values from Figure 3.

the same data in graphic form. Here the increase in rate of dispersion with increase in enzyme concentration is similar to that shown in the papain data. That is, increase in enzyme treatment causes an increase in rate of dispersion with one exception: in the instance of 0.1% pepsin

dosage, the dispersion of the gluten appears to be retarded, pointing, it would appear, to a coagulating effect of this concentration of the enzyme upon the flour gluten. A general conception of enzyme action

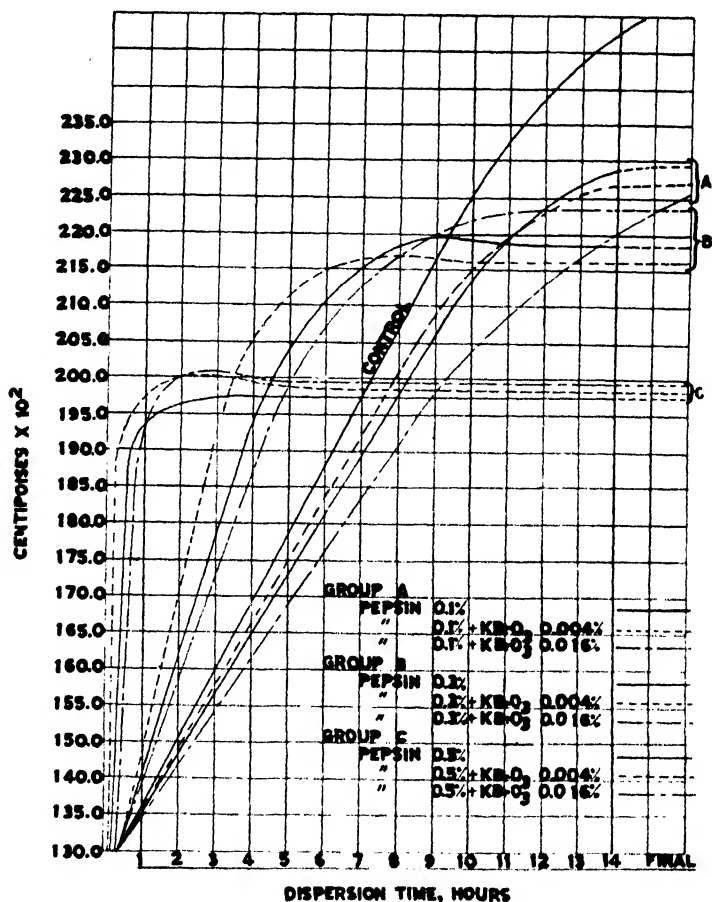


Fig. 3. Effect of pepsin and potassium bromate upon the increase of viscosity with time of dispersion in gluten dispersions.

is that a preliminary coagulation of protein is followed later by disintegration and dispersion. This effect was not noticeable in the case of papain treatments without bromate probably owing to the more drastic action of the latter enzyme upon flour gluten. When we consider the effect of bromate upon pepsin, certain deviations from its effect upon papain are noted. In the case of pepsin, a distinct increase in rate of dispersion is shown for the three concentrations of 0.004%  $\text{KBrO}_3$ , while the heavier concentrations of 0.016%  $\text{KBrO}_3$  repress or inhibit the rate of dispersion. These effects, in the writers' opinion, are due first to an activating effect upon the enzyme exerted by the lower bromate concentration and second to an inhibitory effect upon the enzyme of the higher 0.016% bromate treatment. When com-

paring the results of bromate upon these two enzymes, one must remember that papain is more drastic in its action upon flour gluten than pepsin and the stimulating effects of lower dosages of bromate are not of sufficient magnitude to be revealed in the data dealing with rate of dispersion with the former enzyme. These conclusions are verified mathematically by the values for  $dc/dt$ , which show an initial increase with bromate as compared with the unbromated enzyme treatment followed by a decrease in each instance at the higher bromate concentration. These differences in action of  $KBrO_3$  upon these two enzymes as well as the apparent coagulating effect of low dosages of pepsin are in line with former conclusions reached by the senior author.

Falk and Winslow (1918) also found indications of the possibility of potassium bromate exerting a stimulative action upon the proteolytic enzymes during dough fermentation. These workers found that a concentration of one part bromate in 100,000 to one part in 200,000 appeared to stimulate the digestion of casein by trypsin or pancreatin but when the bromate concentration was increased to one part in 10,000 a slight inhibition of enzymic activity was noted.

The viscosities were calculated from an adaptation of the law of Poiseuille:

$$\eta = \frac{[(d_1)(h) + (\Delta p)d_2]t_2}{hd_3t_1},$$

where  $d_1$  equals density of the liquid as determined by a standardized picnometer,  $h$  equals height of liquid in the viscosimeter,  $\Delta p$  is pressure applied, expressed in cms. of mercury,  $d_2$  is the density of mercury (13.6),  $t_2$  is time of flow at corresponding pressure,  $d_3$  is density of the reference liquid, and  $t_1$  is the time in seconds of the reference liquid. These gluten dispersions were all adjusted to a crude protein content of 2500 mg. per 100 cc. of dispersion. The data obtained on the various liquids using pressure differences in determining the viscosity were calculated as rate of flow ( $1/t \times 10^3$ ),  $t$  being expressed in seconds; viscosity (in centipoises)  $\times 10^2$ ; and viscosity/ $t \times 10^2$ . The latter quantity was computed as a test of the experimental precision of the viscosity tests. In the construction of Figures 4 and 5 the entire set of results obtained was used to obtain as true a picture of the relationships involved as possible.

The data derived from the  $KBrO_3$  treatments with application of pressure were not depicted graphically, because of difficulties in presenting the slight differences obtained between the bromated and unbromated doughs. Differences of little significance were found between the bromated and the unbromated dough gluten dispersions,



although in the instances of 0.004% papain the lower concentration of bromate appeared to increase the rate of flow.

The data relating to rate of flow, as influenced by pressure and presented in Figure 4, show that water has the highest increase in rate of flow with increase in pressure. From the papain data it is apparent that papain significantly increased the rate of flow of the dispersed

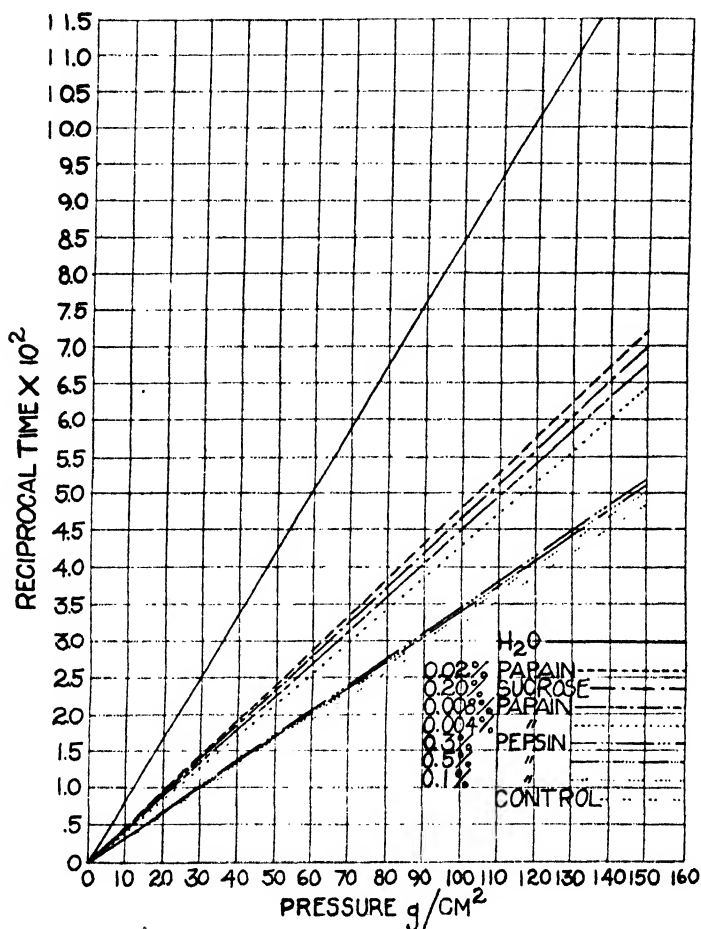


Fig. 4. Effects of pressure upon rate of flow in various liquids and dough gluten dispersions. Data show no evidence of plastic flow. Curves apparently all pass through point of origin.

gluten prepared from doughs to which this enzyme was added. This effect increased with increasing concentration of enzyme. It is also apparent that pepsin increased the rate of flow of dough gluten dispersions in contrast to the untreated dough dispersions, but this effect was much less than in the case of papain and points to fundamental differences in the mode of attack of these enzymes upon flour gluten. The differences shown for the various concentrations for pepsin are scarcely significant. As these curves all pass through the point of

origin, it is evident that plastic flow does not exist and we have true viscous flow. The data depicted in Figure 5 show quite clearly the effect of the two proteolytic enzymes in lowering the viscosity with pressure when the dispersion of the gluten has apparently reached a constant or final value. It will be noticed that the untreated control dough has the highest viscosity curve, followed in order of decreasing viscosity by 0.1%, 0.3%, and 0.5% pepsin respectively.

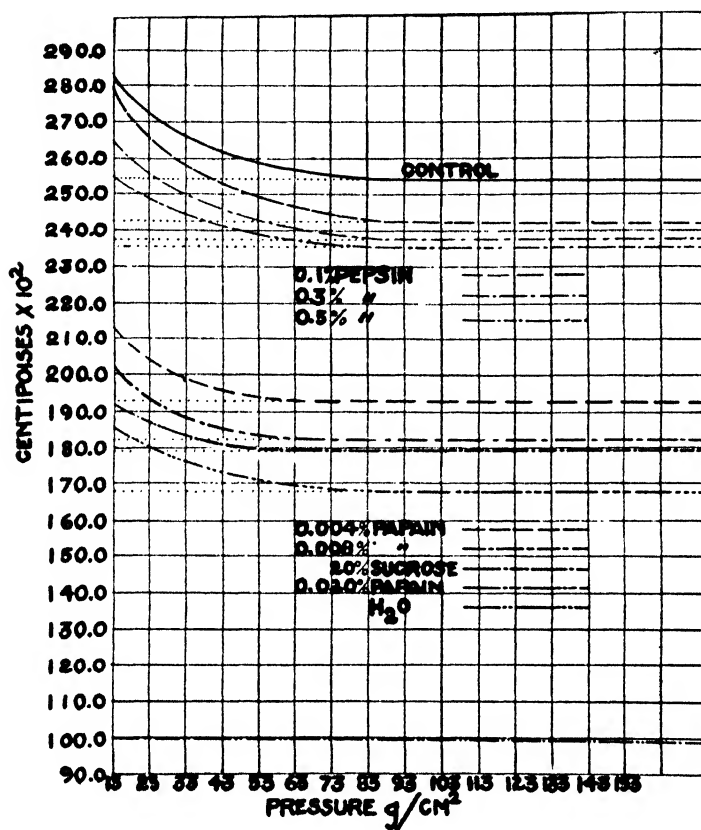


Fig. 5. Effect of pressure upon viscosity of the various gluten protein dispersions including 20% sucrose and redistilled  $H_2O$  for comparison.

The papain curves all show markedly lower viscosities than the curves already mentioned and decrease in order of increasing papain treatment. These results apparently indicate that the structure of the protein micelle has been substantially altered by these two enzymes, especially by papain. It is extremely probable that the size of the protein micelle has been reduced. This particle size reduction appears to be particularly marked in the case of papain and accordingly this enzyme must cleave the gluten complex into smaller portions, whereas the action of pepsin does not progress to nearly the same

degree and particles formed in the instance of this enzyme are much larger in size. It is accordingly probable that the fundamental bonds uniting the various portions of the gluten complex have not been attacked to any extent.

From the viscosity pressure relationships shown in Figure 4, it is evident that water is the only liquid which gives a linear relationship, the other curves being distinctly curvilinear over the initial pressure range. From the equation of Poiseuille ( $\eta = \pi p R^4 / 8lv$ ) it follows that a decrease in the effective radius  $R$  of the capillary will cause a decrease in viscosity. If a decrease of a very small magnitude were effected through the agency of the initial increment of pressure, the result would be an apparent fall in viscosity followed by a constant value for the relationship of this variable with pressure. The final outcome of such a phenomenon would be a curve showing an initial inflection, then shading off into a straight line. It is difficult to picture such a situation arising, however, in the instance of 20% sucrose solution. It was also found that viscosity measured with capillaries of different bores gave the same type of curve when viscosity was plotted against pressure, and this evidence would appear to be against the theory of effective radius change, inasmuch as the magnitude of the change would vary for different capillary radii.

A more plausible explanation is by means of the following considerations. The size of the meniscus of the liquid is changed as the liquid leaves the upper capillary and enters the bulb of the Ostwald pipette. Turbulent flow probably occurs at first with application of pressure to the system, to be later replaced by laminar flow as the pressure is further increased. Resistance is also likely to occur at the lower end of the capillary, which is submerged in the liquid contained in the lower portion of the pipette. The sum total of these effects would be to cause a preliminary loss of energy from the system. This effect would correspond to a decrease in pressure, with apparent decrease of viscosity. As the pressure is further increased these effects are overcome and a linear relationship then results between viscosity and pressure. Liquids with relatively low surface tension, such as water, will not show this effect.

Part of the data were recalculated in terms of viscosity/time and these values are shown in Figure 6. A linear relationship should represent the relationships between the variables shown. A departure from linearity, or a scattering of the dots, would indicate a degree of experimental error or lack of precision in the work. It will be noted from a careful inspection of this figure that the dots do fall very closely on a straight line and that the projection of this line would pass very nearly through the origin. One or two of the variables, especially with

the 20% sucrose solution, do show some displacement from the straight line, which passes through the majority of the points. In a number of cases these dots fell together, and it was therefore necessary to displace several of the points somewhat in order to place them on the figure. This of course would cause a slight distortion in the general appearance of the curve.

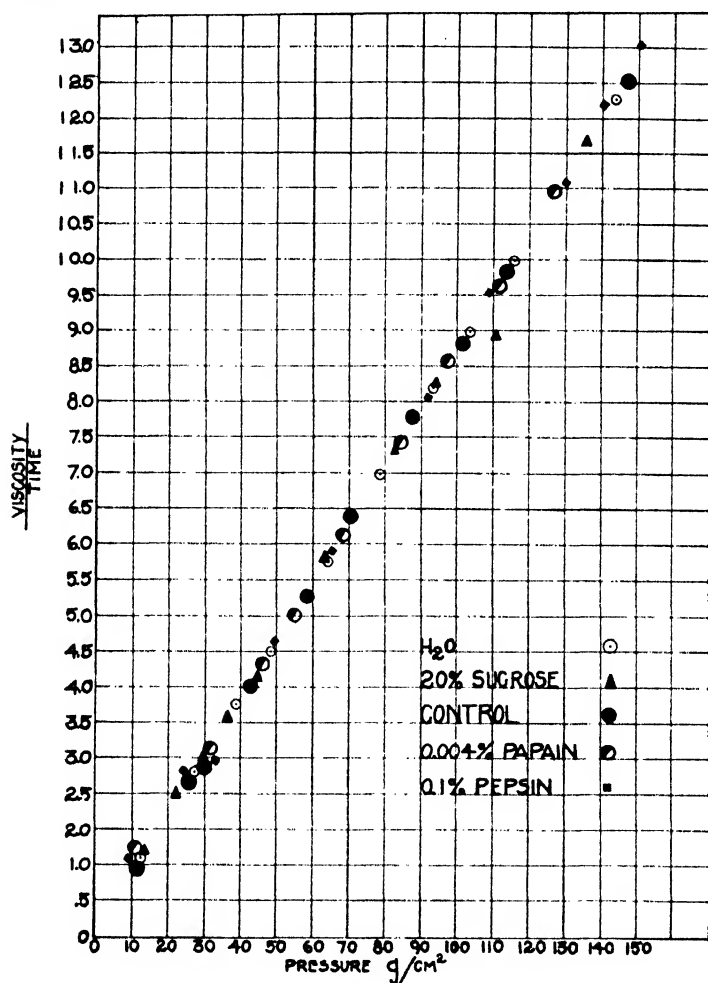


Fig. 6. Relation of the ratio, viscosity/time of flow, to pressure. This is a test of experimental precision and in this instance shows little evidence of any substantial error in viscosity determinations.

Table III contains the data obtained by fractionating the gluten protein from the dispersions following the determination of the relationships between pressure, rate of flow, and viscosity. The effect of increasing the papain dosage is shown in a marked fall in the quantity of protein removed from the dispersion, and corresponds with former results published by the senior author. Pepsin, on the other hand, shows little effect upon the protein fractionated. These results are

TABLE III

EFFECT OF INCREMENTS OF  $\text{KBrO}_3$  UPON THE QUANTITY OF PROTEIN FRACTIONATED FROM 10% SODIUM SALICYLATE DISPERSIONS OF GLUTEN WASHED FROM DOUGHS MADE UP WITH ADDITIONS OF PAPAIN AND PEPSIN<sup>1</sup>

Results expressed in mg. protein per 100 cc. dispersion<sup>2</sup>

Treatment	$\text{KBrO}_3$ %		
	0.0	0.004	0.016
Control			
(No enzyme added)	1630	—	—
Papain 0.004%	997	986	835
Papain 0.008%	648	658	638
Papain 0.02%	392	319	361
Pepsin 0.1%	1590	1602	1642
Pepsin 0.3%	1613	1545	1607
Pepsin 0.5%	1596	1585	1516

<sup>1</sup> Results show quantity of protein precipitated by 6 cc. of concentrated  $\text{MgSO}_4$  solution.

<sup>2</sup> Dispersions adjusted to a protein concentration of 2500 mg. per 100 cc.

depicted graphically in Figure 7. These papain relationships are similar to former data published by Harris (1938) using lower gluten protein concentrations (not on a definite protein basis) in the dispersion. These fractionation results correspond roughly to the final viscosities of these dispersions and offer further evidence of the comparative effects of papain and pepsin upon particle size in these dispersions. As papain concentrations increase, particle size decreases and less protein is removed by the fractionation procedure. In the case of pepsin, little effect upon particle size occurs and accordingly little change is evident in the fractionation value.

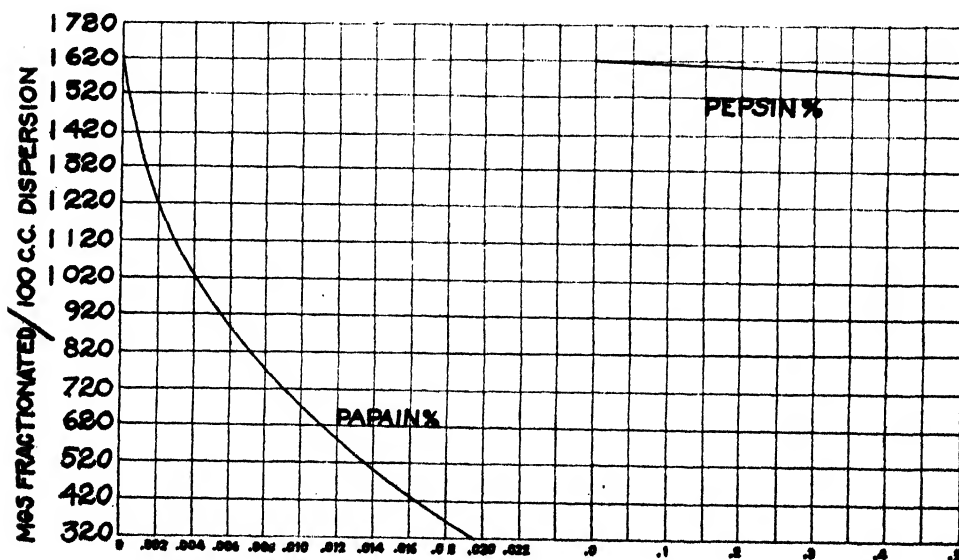


Fig. 7. Comparative effects of increments of papain and pepsin upon the quantity of protein fractionated from dough gluten dispersions.

The effect of  $\text{KBrO}_3$  upon fractionation is also shown in Table III and is represented graphically in Figure 8. In discussing first the effect of bromate upon papain, it appears that the lowest concentration of enzyme, namely 0.004%, is activated by bromate as the quantity of protein removed is diminished by the bromate, which corresponds

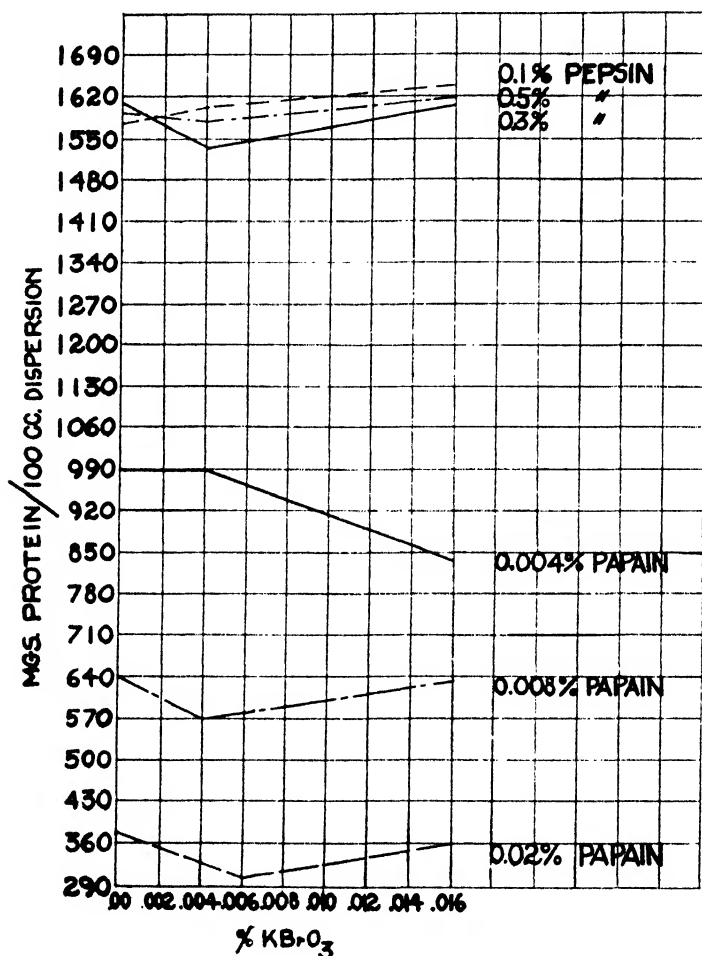


Fig. 8. Comparative effects of increments of potassium bromate upon various concentrations of papain and pepsin flour doughs as registered in mgs. of protein fractionated from dough gluten dispersions.

to the effect of increasing the enzyme concentration, as shown in Figure 7. When the papain concentration is increased, however, the lower dosage of bromate activates, while the heavier dosage represses. Bromate appears to have little effect upon pepsin, and this result would be expected with pepsin, considering that this enzyme does not appear to change the particle size materially in these dispersions.

### Summary and Conclusions

Glutens were washed from a large number of doughs prepared from a hard red spring wheat flour. Various quantities of papain and pepsin were added to these doughs. Potassium bromate was also used in addition to the enzymes in increments of 0.004% and 0.016%. Control doughs were run for comparative purposes. The glutens were washed immediately after the doughs were mixed and dispersed in 10% sodium salicylate solution. The rate of dispersion was determined by viscosity measurements.

The results obtained indicated a marked rise in viscosity with time of dispersion. This rise was distinctly greater in most instances in the dispersions prepared from doughs treated with enzymes, although there was some evidence that a low concentration of pepsin decreased the rate of dispersion, probably owing to a slight coagulation of gluten protein. Bromate retarded dispersion in the papain-treated doughs, but increased the rate for pepsin when present in 0.004% concentration. A further investigation was conducted, using a higher concentration of dispersed gluten and adjusting the final concentration to 2500 mg. of protein per 100 cc. Viscosities were then run on these dispersions with suitable pressure variations. The quantity of protein fractionated by adding 0.6 cc. of concentrated  $MgSO_4$  solution to 10 cc. of the colloidal solution was then found.

The data obtained by the method showed that apparently viscous flow was present in all the dispersions. Papain had a greater effect than pepsin in increasing the rate of flow and in decreasing the viscosity, pointing it would appear to the production of smaller particles caused by differences in protein cleavage by the two enzymes. Bromate did not show any marked effect in this instance. The fractionation data also gave evidence of papain activity in reducing particle size with increasing enzyme concentration, while pepsin had little effect. Some evidence of a slight activation of papain at 0.004% concentration by bromate was noted, followed by repression as the papain concentration increased.

### Acknowledgment

The authors wish to express their sincere appreciation of valuable advice contributed by Dr. R. A. Gortner and Dr. Dallas Dedrick regarding the viscosity determinations. Acknowledgment is also made of the aid of John Monge in working up the data and constructing the graphs and of technical assistance from WPA and NYA funds.

### Literature Cited

- Balls, A. K., and Hale, W. S.  
1938 The preparation and properties of wheat proteinase. *Cereal Chem.* **15**: 622-628.  
Bayfield, E. G.  
1933 Report of the subcommittee on the viscosity test for soft winter wheat flours. *Cereal Chem.* **10**: 494-501.

- 1935 Soft winter wheat studies. III. The effect of some factors influencing viscosity and protein. *Cereal Chem.* 12: 1-16.
- Blish, M. J., and Sandstedt, R. M.  
1925 Viscosity studies with Nebraska wheat flours. *Cereal Chem.* 2: 191-200.
- Denham, H. J., Scott Blair, G. W., and Watts, G.  
1927 Notes on the use of Ostwald viscometers for flour suspensions. *Cereal Chem.* 4: 206-220.
- Watts, G., and Scott Blair, G. W.  
1928 Note on the measurement of viscosity in flour suspensions. *Cereal Chem.* 5: 330.
- Falk, I. S., and Winslow, C.-E. A.  
1918 The effect of potassium bromate on enzyme action. *J. Biol. Chem.* 33: 453-462.
- Gortner, R. A.  
1924 Viscosity as a measure of gluten quality. *Cereal Chem.* 1: 75-81.
- Harris, R. H.  
1938 A study of the effect of proteoclastic enzymes upon wheat gluten solubility in sodium salicylate solutions. *Cereal Chem.* 15: 690-707.  
1939 A study of gluten protein fractionation from sodium salicylate solution. III. The effect of proteolytic enzymes. *Cereal Chem.* 16: 78-88.
- Johnson, A. H.  
1927 Wheat and flour studies. X. Viscosity of flour-water suspensions. *Cereal Chem.* 4: 87-126.  
1931 Wheat and flour studies. XVII. Factors influencing the viscosity of flour-water suspensions. IV. The effect of extraction with solutions of the potassium and sodium halides. *Cereal Chem.* 8: 39-43.
- and Green, J.  
1930 Wheat and flour studies. XVI. The effect of yeast fermentation on the viscosity of flour-water suspensions. *Cereal Chem.* 7: 117-132.
- and Herrington, B. L.  
1928 Wheat and flour studies. XII. Factors influencing the viscosity of flour-water suspensions. II. Effect of hydrogen-ion concentration during extraction or digestion period. *Cereal Chem.* 5: 14-44.  
1928a Wheat and flour studies. XIV. Factors influencing the viscosity of flour-water suspensions. III. Effect of small quantities of carbon dioxide in water used for the extraction of electrolytes. *Cereal Chem.* 5: 105-116.
- — and Scott, S. G.  
1929 Wheat and flour studies. XV. The use of the viscometric method for measuring the proteoclastic activity of flours. *Cereal Chem.* 6: 182-196.
- Jong, H. G., Bungenberg de, and Klaar, W. J.  
1930 Contribution to the knowledge of colloid chemistry of the gluten. II. *Cereal Chem.* 7: 222-244.
- Landis, Q., and Frey, C. N.  
1938 A sensitive method for the determination of proteolytic activity. *Cereal Chem.* 15: 91-101.
- Mangels, C. E.  
1934 Varietal and regional variation in properties of wheat starches. *Cereal Chem.* 11: 571-585.  
1936 Varietal and regional variation in durum wheat starches. *Cereal Chem.* 13: 221-233.
- and Bailey, C. H.  
1933 Relative viscosity of wheat starches. *Ind. Eng. Chem.* 25: 456-460.
- Morgan, Ralph W.  
1924 A practical application of the viscosimeter to the mill. *Cereal Chem.* 1: 288-292.
- Rich, C. E.  
1932 The relationship between viscosity, total protein content, ash content, and baking strength of experimentally and commercially milled flours from western Canadian hard red spring wheat. *Cereal Chem.* 9: 535-559.
- Rose, R. C., and Cook, W. H.  
1935 Viscosity of gluten dispersed in alkali, acid and neutral solvents. *Can. J. Research* 12: 63-81.
- Scott Blair, G. W., Watts, G., and Denham, H. J.  
1927 Effect of concentration on viscosity of flour suspensions. *Cereal Chem.* 4: 63-67.



Sharp, Paul F.

1926 Wheat and flour studies. V. Plasticity of simple flour-in-water suspensions. *Cereal Chem.* 3: 40-56.

— and Gortner, R. A.

1923 Viscosity as a measure of hydration capacity of wheat flour and its relation to baking strength. *Minn. Agr. Exp. Sta. Res. Bul.* 19.

1924 The physico-chemical properties of strong and weak flours. VIII. Effect of yeast fermentation on imbibitional properties of glutenin. *Cereal Chem.* 1: 29-37.

Vogel, H., and Bailey, C. H.

1927 A study of durum wheats. *Cereal Chem.* 4: 136-149.

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## A COMPARATIVE STUDY OF SOME PROPERTIES OF DRIED GLUTENS PREPARED FROM VARIOUS TYPES OF WHEAT

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(Received for publication July 13, 1939)

Several years ago the writer, in collaboration with H. N. Bergsteinson,<sup>1</sup> built up the protein content of a soft-wheat flour dough by additions of freshly washed wheat gluten prepared from hard red spring wheat flour. The gluten was reduced to small shreds by hand and added to the flour and other dough ingredients in the mixing bowl. It was then incorporated into the dough by careful mixing. The moisture and protein content of the gluten were determined and from these data the final protein level of the resultant blend could be calculated. The levels used were 12.1%, 16.7%, and 21.3%, built up upon a 7.5% protein flour. These values are on a 13.5% moisture basis. The baking formula used was the malt-phosphate-bromate, superimposed upon the A.A.C.C. standard basic. Photographs of the resultant four loaves are shown in Figures 1 and 2, which depict the size, external appearance, and interior characteristics of these loaves. It is quite evident that marked improvements in these loaf attributes resulted from the addition of wet crude gluten to the soft-wheat flour. It is very probable that further differentiation, among the loaves, especially in volume, would have been secured if larger increments of  $\text{KBrO}_3$  had been used for the higher protein levels.

Aitken and Geddes (1938) devised a method whereby the protein contents of flours could be adjusted to any desired level through the addition of dried and ground gluten, thereby obviating possible difficulties in properly incorporating wet crude gluten in the dough and facilitating the determination of the quantity required for any desired

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protein level. In addition, the danger of changes in wheat gluten properties during the time required for moisture and protein determinations was eliminated. These workers used a low drying temperature of 32°C. and a rapid air flow through a falling humidity gradient. The gluten was then reduced to flour-like fineness before using. The addition of such gluten flour to a series of weak, intermediate, and strong world wheats to equalize their protein contents resulted in a marked improvement in flour strength but the crumb color was impaired. From their results the authors concluded that the majority of world wheats possessed glutens of similar character, and their varying



Fig. 1. External appearance of loaves baked from blends of hard red spring wheat gluten and soft wheat flour. Gluten used in original undried form.



Fig. 2. Internal appearance of loaves baked from blends of hard red spring wheat gluten and soft wheat flour. Gluten used in original undried form.

strength characteristics were due to protein-content differences. Some wheats of English, German, and Italian origin are deficient in baking quality owing to lack of satisfactory protein content, combined with a weak and unsatisfactory nature of the gluten. It was noticed that the degree of dispersion in 0.05*N* acetic acid had been somewhat lowered as a result of drying. In view of the results obtained by Aitken and Geddes with dried gluten, the author decided to investigate the effects of additions of gluten flour prepared from different classes and varieties of American wheats and prepared, as far as feasible, by their method. It was decided to determine the effects, as far as possible, of the drying

process upon the properties of these glutens using gluten dispersions in 10% sodium salicylate solution as described by me in former publications (1938, 1939), and by Harris and Johnson (1939, 1940).

### Material and Methods

Fourteen lots of flour containing both commercially and experimentally milled samples from different types and varieties of wheat were included in this investigation. This series covered as wide a range in baking strength and genetic variability as it was possible to secure, and should, therefore, yield pertinent information regarding the relationships of gluten properties to these two variables.

Durum wheats were also included, because of their commercial importance in North Dakota, with the aim of obtaining information upon the gluten characteristics of these wheats. A soft-wheat flour commercially milled from Pacific Coast wheat containing 7.5% protein was used as a basic flour upon which the various gluten flours were superimposed. The malt-phosphate-bromate formula <sup>2</sup> was used throughout the baking test. The original flours were also baked by this formula.

In preparing the glutens, the flours were mixed into a stiff dough with 0.1% sodium phosphate solution (pH 6.8) in the Hobart-Swanson, and allowed to stand a short time at room temperature under tap water. The glutens were washed under a small stream of sodium phosphate solution, covered with the same solution for approximately 15 to 30 minutes, then divided into two- to three-gram portions and placed upon sheets of waxed paper for drying. The sheets of gluten were placed in a fermentation cabinet equipped with a temperature control, a Bahnson humidifier, and an electric fan to insure thorough air circulation. The drying temperature was held at  $32 \pm 2^\circ\text{C}$ . and the humidity gradient was gradually lowered manually.

At the end of 24 hours, the gluten pieces appeared to be sufficiently dried and were removed from the waxed paper and reduced to flour in the micro mill. Moisture, ash, and protein determinations were run on these glutens. The quantity of dried gluten required to raise the protein level of the soft-wheat flour to 14.0% (13.5% moisture level) was calculated in each case. The bakings were made in triplicate and the results averaged. The doughs were mixed for three minutes in the Hobart-Swanson with sufficient water to produce normal dough consistency. The dried gluten appeared to be entirely incorporated in the dough and no streaks or dark spots were visible in the baked loaves. In addition samples of wet crude gluten, the dried gluten

<sup>2</sup> This formula contained 0.3% diastatic malt, 0.1% ammonium phosphate, and 0.001% KBrO<sub>3</sub> superimposed upon the A.A.C.C. basic formula which contained 5% sucrose instead of the customary 2.5%.

powder, and gluten reformed from the dried gluten by suitable additions of sodium phosphate solution were dispersed in sodium salicylate, the protein content of the dispersions was adjusted to a concentration of 1400 mg. per 100 cc., and the viscosity<sup>3</sup> and protein fraction were determined. In this way a comparison was obtained between the three gluten dispersions in regard to these properties, as well as with respect to apparent solubility in the dispersing agent, visual appearances of the dispersion, etc., in order to permit conclusions regarding any changes induced in the washed gluten by the drying method.

### Discussion

A description of the flours used in this investigation, with ash, crude protein, and baking data, is presented in Table I. The results

TABLE I  
PROTEIN, ASH AND BAKING DATA ON FLOURS USED IN THIS INVESTIGATION<sup>1</sup>  
(Arranged in order of increasing flour protein)

Sam- ple No.	Type of flour	Ash	Crude protein (N×5.7)	Loaf vol- ume	Color	Tex- ture
		%	%	cc.	%	%
1	Pacific Coast soft	0.32	7.5	359	96.0	89.3
2	Soft red winter	0.48	8.5	469	92.5	94.0
3	Hard red winter (Chiefkan)	0.48	10.5	525	92.5	93.5
4	Yaroslav emmer	0.58	12.2	440	91.0	93.0
5	Hard red winter	0.51	12.3	616	93.5	92.5
6	Hard red spring (Nordhaugen)	0.40	12.6	606	94.5	92.0
7	Durum (Monad)	0.72	12.7	525	94.5	93.0
8	Durum (Mindum X Vernel)	0.66	13.0	417	91.0	93.0
9	Hard red spring (commercially milled)	0.51	13.1	695	94.5	93.0
10	Durum (Pentad)	0.69	13.4	465	90.0	93.0
11	Hard red spring (Thatcher)	0.37	13.5	695	94.5	92.5
12	B & S duster bottom, mill-stream	1.74	16.6	570	89.0	90.0
13	3rd break, mill-stream (com- mercially milled)	0.66	17.0	692	92.0	91.0
14	Durum (Mindum)	0.88	17.1	465	90.0	93.0
15	5th break, mill-stream (com- mercially milled)	1.35	19.4	645	89.0	90.0
Average			13.7	559	92.0	92.4

<sup>1</sup> On 13.5% moisture basis.

are arranged in order of increasing protein content, inasmuch as this factor is an important index of flour strength. It is evident from this table that a wide range of strength existed among these flours. The four principal wheat types grown in the continental United States are included, as well as several commercial mill-stream flours of relatively high protein content. A number of durumms and an emmer were

<sup>3</sup> Viscosities were determined by an Ostwald pipette at 25 C. Densities were measured with a pycnometer standardized against redistilled water.

included in the experimental material to furnish information in respect to the gluten properties of these wheats and their relationships to baking strength. The protein content ranged from 8.5% to 19.4%, a difference of 10.9%, while the loaf volume varied from 417 cc. to 695 cc. Flour No. 1, which had the lowest protein content and loaf volume, was used as a basic flour to which the dried gluten was added. Several of the durum wheat flours gave loaves of inferior volume, as would be expected, and substantially lowered the average loaf volume for this set of flours. These durum results also tended to lower the relationship between protein content and loaf volume. A large variability in flour ash existed among the various samples of flour.

In Table II are shown the comparative data obtained from the blends of soft-wheat flour and dried gluten. More variability was

TABLE II  
PROTEIN CONTENT AND BAKING DATA ON BLENDS OF SOFT-WHEAT  
FLOUR AND DRIED GLUTEN

No. of flour from which gluten was prepared	Crude protein (N×5.7)	Loaf volume	Color	Texture	Variation of loaf vol. from original
	%	cc.	%	%	cc.
1 Control, no gluten added	7.5	359	96.0	89.3	—
2	13.7	467	95.0	95.0	+ 2
3	13.5	513	93.0	94.0	+ 12
4	13.8	488	92.0	94.0	- 48
5	13.9	494	95.3	96.3	+122
6	14.0	481	95.3	94.3	+125
7	13.3	476	94.0	94.0	+ 49
8	13.5	492	92.0	93.1	+ 75
9	14.0	469	95.3	93.7	+226
10	14.0	482	93.0	94.0	- 17
11	14.1	453	95.0	95.0	+242
12	13.7	397	92.0	94.0	+173
13	13.5	456	94.0	95.0	+236
14	14.0	474	94.3	94.7	- 9
15	13.5	482	94.0	94.0	+163
Average of blends	13.8	473	93.9	94.4	+ 96.5

evident in the protein contents of these blends then was desired, but it is probable that this had little effect upon the final results obtained. The loaf volumes did not differ greatly from the general level except in the instance of Chiefkan and the B and S duster bottom flour blends. Why Chiefkan gluten yielded the largest loaf is not apparent to the writer, and the loaf size is probably misleading as far as its general character is concerned. The relatively poor showing of B and S duster

bottom flour can doubtless be explained by the lower quality of its gluten, as this particular mill-stream is one of the poorest-quality streams in the mill set-up. These data support the conclusion of Aitken and Geddes (1938) that the great majority of bread flours milled from sound wheats differ mainly in baking strength on account of protein quantity differences.

The durum-wheat-gluten blends showed up remarkably well as compared with the loaf volumes of these flours shown in Table I. The greatest differences in loaf volume in the two sets of data are shown by the stronger flours, while the durum flours gave the smallest differences, and in several instances yielded smaller loaves when baked by themselves than when their dried glutens were blended with the soft-wheat flour. It will also be noticed that the loaves baked from the blend of soft-wheat flour and gluten were, on the average, of better color and texture than the average loaves produced from the flours themselves. This is contrary to the findings of Aitken and Geddes, but may be explained in the present instance by the presence of the durum flours which yield a loaf of distinctly poor color when baked alone. The degrading effect upon color of the durum glutens was masked by the white color of the soft-wheat-base flour in the blends. The same effect is true for the break and duster mill-stream flour glutens which, when baked into loaves with the soft wheat flour, were nearly equal in color to those produced from the spring-wheat-gluten blends. The texture of the blended flour loaves was also superior.

It will be noticed that the average protein content of the original series of flours and that of the blended series were practically identical. The average loaf volumes, on the other hand, were 86 cc. higher for the flours, thus showing that the addition of dried gluten to the soft-wheat flour did not bring the soft-wheat-flour blends to the same level of baking strength as the original flours. This effect may possibly be explained by the presence of poor-quality gluten in the soft winter wheat which lowered the strength of the blend, especially in the case of a superior flour whose gluten quality had now been decreased by the soft wheat gluten contained in the blend. No gluten was washed and blended from flour No. 1, principally because of the lack of a sufficient quantity of this flour for both blending and gluten preparation. The results obtained from flour No. 2, which contained only 1% more protein than flour No. 1, showed no evidence of lower quality in its gluten than the other flours. Comparative loaf-volume results are reported graphically in Figure 3. The gluten-blend loaf volume shown for flour No. 1 is the average value obtained when it was blended with the glutens from all the other flours.

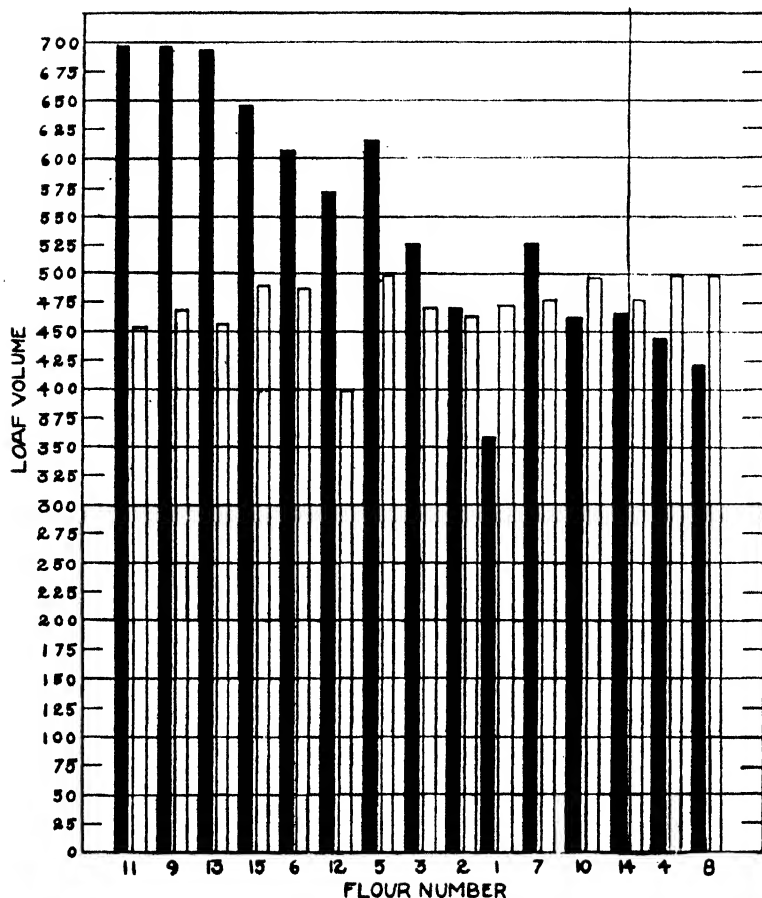


Fig. 3. Loaf volumes of the flours compared with loaf volumes of the dried-gluten and soft-wheat blends. Black bars represent flour loaf volumes; white bars represent gluten-blend loaf volumes.

In Table III are shown the moisture, ash, and protein content of the dried and ground glutens. A substantial range in ash is evident, corresponding roughly to a similar variability in the flour ash. It is evident that a large proportion of the flour ash is carried along with the gluten, although a small percentage of the gluten ash may consist of sodium phosphate absorbed from the washing solution. Some variability in protein content is also noticeable. No relationship between the original flour protein and the protein content of the washed crude gluten is to be noted.

The viscosity and fractionation results obtained on the three series of gluten dispersions are shown in Table IV. The *A* series was prepared from the original wet ~~crude~~ freshly washed gluten. Series *B* was made from wet gluten reformed by the addition of suitable quantities of sodium phosphate solution to the dried gluten. Series *C* was prepared by adding a proportionate quantity of dried gluten directly

TABLE III  
MOISTURE, ASH, AND PROTEIN CONTENTS OF THE DRIED GLUTENS

Flour No.	Moisture	Ash <sup>1</sup>	Crude protein <sup>1</sup> (N×5.7)
	%	%	%
2	6.1	0.78	64.3
3	6.7	1.17	63.2
4	6.7	1.06	65.5
5	6.8	0.77	66.9
6	6.3	0.55	68.9
7	7.5	1.38	67.7
8	7.5	1.29	63.2
9	5.9	0.73	67.4
10	7.1	1.38	64.2
11	7.1	1.38	64.2
12	5.6	1.26	65.4
13	5.6	0.82	64.8
14	6.4	1.46	70.0
15	5.3	1.26	65.4

<sup>1</sup> 13.5% moisture basis.

TABLE IV  
VISCOSITY AND QUANTITY OF PROTEIN FRACTIONATED FROM THE THREE SERIES OF GLUTEN DISPERSIONS <sup>1</sup>

Flour No.	Viscosity—Centipoises×10 <sup>3</sup>			Protein fractionated per 100 cc. dispersion		
	Series A	Series B	Series C	Series A	Series B	Series C
				<i>mgs</i>	<i>mgs</i>	<i>mgs</i>
2	200.1	198.7	199.4	632	611	506
3	181.2	179.4	186.0	586	522	506
4	190.2	186.9	183.4	768	739	575
5	211.7	207.8	204.3	739	730	595
6	211.8	198.5	187.9	670	622	335
7	186.2	179.0	175.6	604	645	451
8	185.6	183.8	186.9	566	946	508
9	210.3	204.8	188.4	691	688	410
10	190.9	185.9	185.5	698	686	549
11	210.5	200.9	188.1	659	620	410
12	207.7	199.8	183.0	723	616	513
13	207.2	195.3	205.4	659	570	490
14	192.7	185.0	183.7	534	452	374
15	207.0	195.7	182.9	534	609	489
Means	199.5	193.0	188.6	647.4	646.9	479.4

<sup>1</sup> Adjusted to a concentration of 1400 mg. protein per 100 cc.

to 10% sodium salicylate. The protein concentrations, as pointed out in the table footnote, were adjusted to 1400 mg. per 100 cc. While there is evidence of considerable variation in the corresponding values of series A and B the quantity of protein fractionated is approximately the same for each series, while for series C these results are substantially lower.



These results would lead one to infer that the gluten dispersions prepared from the reformed wet crude gluten more nearly resemble the original wet crude gluten dispersions than would the dispersions prepared from dried gluten without the preliminary addition of water. The mean viscosity results, however, steadily decreased from *A* through *B* to *C*. Series *C* did not resemble series *A* so closely as series *B*. The dried and reformed glutens did not appear to be as extensible and elastic as the undried glutens; they were also somewhat darker in color. They did, however, appear to disperse as readily as the undried glutens in sodium salicylate. This observation is at variance with the observations of Aitken and Geddes (1938), who noted that dried gluten dispersed less in 0.05*N* acetic acid than the corresponding freshly prepared gluten and caused these workers to conclude that the drying process had slightly altered the gluten. Similar alterations had apparently taken place to some extent in the present investigation, although not to the extent of seriously affecting the viscosity and fractionation results of the dispersions prepared from reformed, dried gluten.

When the dried gluten powder was added to the sodium salicylate solution, a distinct difference in properties became evident. The appearance of the dispersions was quite different, flecks of heavily hydrated gluten particles apparently being present in each dispersion. These larger particles were then removed when the dispersion was centrifuged, leaving the more highly dispersed portion of the gluten in the liquid. Some evidence of this is found in the distinctly lower viscosity and fractionation results, indicating the presence of smaller particles as compared with the other two series of dispersions. It is probable that this difference was caused by the action of the sodium salicylate itself upon the dried gluten particles. Table V contains the correlation coefficient calculated from the comparative data presented in Table IV. A high correlation is to be noted between the viscosities of the series *A* and *B* dispersions.

TABLE V  
CORRELATION COEFFICIENTS COMPUTED FROM THE VISCOSITY  
AND FRACTIONATION DATA

Variables correlated		
<i>X</i>	<i>Y</i>	<i>r<sub>xy</sub></i>
Viscosity, Series <i>A</i>	Viscosity, Series <i>B</i>	+ .9411
Viscosity, Series <i>A</i>	Viscosity, Series <i>C</i>	+ .4618
Fractionation value, Series <i>A</i>	Fractionation value, Series <i>B</i>	+ .2576
Fractionation value, Series <i>A</i>	Fractionation value, Series <i>C</i>	+ .3979

### Summary and Conclusions

Glutens were washed from a series of 14 flours which comprised samples milled from hard red spring, hard red winter, soft red winter, and durum wheats. These glutens were dried at a constant temperature, ground into flour-like consistency, and the moisture, ash, and protein contents determined. Calculated additions of these gluten powders were then made to a soft-wheat, low-protein flour to bring the final protein level to the neighborhood of 14%. Bakings were done in triplicate and the results averaged.

The glutens prepared from the hard red spring wheats markedly increased the loaf volumes of the blends, as did one sample of gluten from hard red winter wheat. The soft-winter-wheat gluten was negligible in effect, while the durum-wheat glutens in several instances decreased the loaf volume. The texture and color scores of the blends were superior to the average scores of the loaves from the original flours.

Three series of gluten dispersions were also prepared. Series *A* was from the original wet crude gluten, series *B* from the reformed dried gluten, and series *C* from the dried gluten powder added directly to the sodium salicylate. The resultant dispersions were adjusted to a protein concentration of 1400 mg. per 100 cc. The viscosity was determined and the gluten protein fractionated by suitable additions of  $\text{MgSO}_4$  solution. The results obtained showed good agreement on the average between series *A* and series *B* as far as the fractionation data were concerned, but series *B* had a lower viscosity on the average than series *A*, probably indicating some alteration in gluten properties. Series *C* had a lower average viscosity and quantity of protein fractionated than series *B*. When compared with series *A* the viscosity differences were still larger, pointing apparently to the presence of smaller gluten particles in series *C*. This conclusion was further borne out by the outward appearance of the three series of dispersions.

In conclusion it may be stated that the method of drying washed crude gluten prepared by Aitken and Geddes (1938) has proved entirely feasible, although there is some evidence of alteration in the properties of the dried glutens. Substantial improvements in loaf volumes were obtained when hard-wheat glutens were added to the soft-wheat flour while the durum glutens tended to lower the loaf volume. Average color and texture scores were improved by the addition of the dried gluten.

### Acknowledgments

The author wishes to acknowledge the technical assistance of John Monge in obtaining and assembling the data. Acknowledgment is also made of technical assistance from the NYA funds.

## Literature Cited

- Aitken, T. R., and Geddes, W. F.  
1938 The effect on flour strength of increasing the protein content by addition of dried gluten. *Cereal Chem.* 15: 181-196.
- Harris, R. H.  
1938 A study of the effect of proteoclastic enzymes upon wheat gluten solubility in sodium salicylate solutions. *Cereal Chem.* 15: 690-707.  
1939 A study of gluten protein fractionation from sodium salicylate solution. III. The effect of proteolytic enzymes. *Cereal Chem.* 16: 78-88.
- and Johnson, John Jr.  
1939 A study of gluten protein fractionation from sodium salicylate solution. Part IV. Effect of proteolytic enzymes, as influenced by class of wheat. *Cereal Chem.* 16: 279-289.  
1940 A study of the effects of proteolytic enzymes and  $KBrO_3$  upon the viscosity and allied properties of dispersions of hard red spring wheat gluten. *Cereal Chem.* 17: 203-222.

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**THE APPLICATION OF VISCOSITY AND FRACTIONATION  
MEASUREMENTS TO THE DETERMINATION OF  
DIFFERENCES IN GLUTENS PREPARED FROM  
VARIOUS CLASSES OF WHEAT**

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(Received for publication August 11, 1939)

Cereal technologists have endeavored from time to time to definitely associate wheat and flour "strength" with the physical and chemical properties of gluten. Such factors as the relative proportions of the gluten proteins present, the viscosity of flour-water suspensions, plasticity and extensibility of doughs and glutens, etc., have been studied by various investigators. As some of these researches were conducted upon doughs and flour-water suspensions which contained large quantities of starch and other non-gluten substances, it seems probable that studies performed with gluten itself as investigational material might yield more pertinent information. Gluten is comparatively easy to prepare, and can be readily dispersed in sodium salicylate solution, as demonstrated by Cook and Alsberg (1931) and Cook and Rose (1934). The properties of the gluten dispersion can then be studied with respect to gluten quality differences. Harris (1937, 1938, 1939) found that the quantity of gluten protein fractionated from sodium salicylate dispersions varied greatly according to the basic loaf volume of the flours from which the gluten had been prepared and the treatment to which the gluten had been subjected while present in the dough prior to washing out.

Harris and Johnson (1939) investigated four flours representative of the hard red spring, hard red winter, soft red winter and durum

wheat classes, using gluten protein dispersions and measuring the quantity of protein precipitated by  $\text{MgSO}_4$ . The flours were mixed into doughs in the customary manner, suitable increments of papain, pancreatin, and yeast water being added. Substantial differences were found in the amounts of protein fractionated among the different classes of wheat flour, and led the authors to believe that hard red spring wheat flour gluten dispersed in sodium salicylate was of larger particle size than that in winter wheat and durum wheat flour gluten dispersions. The results obtained were not quantitative, however, and could therefore not be regarded as highly conclusive.

Harris and Johnson (1940) applied viscosity as well as fractionation methods to the determination of the comparative effects of papain and pepsin upon the fractionation and viscosity values of gluten dispersions prepared from doughs containing these enzymes. Marked differences in effects upon viscosity, rate of flow, etc., were noted between these enzymes, which appeared to indicate differences in size of the protein particle induced by the action of the two enzymes. Differences in the effect of potassium bromate on the two enzymes were also found. It was therefore thought advisable to study further the properties of gluten dispersions prepared from hard red spring, hard red winter, soft red winter, and durum wheat flours, for the purpose of obtaining additional information regarding size of particles in these colloidal solutions. The most desirable procedure appeared to be the use of a series of gluten concentrations to determine the quantity of protein fractionated by  $\text{MgSO}_4$  and to measure the viscosities of the various dispersions.

### Material and Methods

A number of wheats comprising representatives of the hard red spring and winter, soft red winter, and durum classes were included in this investigation. A sample of the hard winter variety, Chiefkan, was also included for comparative purposes as it is generally considered to be distinctly inferior in baking quality. The hard red spring flour was commercially milled but had not been diastated or bleached in the mill. The wheats were milled into straight-grade flour on the Allis-Chalmers experimental mill, and the flours were analyzed for protein and ash content and baked by the malt-phosphate-bromate method. The glutes were washed from these flours by the method described by the senior author in a former publication and dispersed in 10% sodium salicylate solution. Concentrations of 2%, 3%, 4.5%, 6%, 15%, and 20% gluten in sodium salicylate were used. The dispersion of the gluten, which had been divided into approximately

half-gram pieces, was completed in three or four days, during which period the flasks were vigorously shaken by hand. At the end of the dispersion process, the colloidal solutions were centrifuged to remove the undispersed material, and the nitrogen content determined by a modified Kjeldahl-Gunning method. The viscosities of these dispersions were then ascertained by means of the Ostwald pipette at a temperature of 25° C. These solutions were not adjusted to a constant protein concentration for each increment of gluten, but a correction was made from a plotted curve which yielded essentially the same results. The quantity of protein precipitated from the dispersions by the addition of  $\text{MgSO}_4$  solution to 6% concentration by volume was next determined.

In consideration of results previously obtained by Harris and Johnson (1940) when decided drops in viscosity were obtained with small increases in the pressure under which the dispersions were forced through the pipette, different pressures were also used in determining the viscosities of these dispersions. It was decided to use the Ostwald pipette throughout for this experiment, although when the higher pressures were used with the less viscous dispersions the time of flow was too short to obtain the most precise results. Another slight discrepancy was introduced by the reading of the mercury manometer in the low-pressure regions. In spite of these probable sources of error, however, the values obtained showed very definite trends. A description of the apparatus used in these determinations was published by the authors in a former paper (Harris and Johnson, 1940).

### Discussion

In Table I is shown a description of the flours used in this research, with flour protein, flour ash, and data obtained with the use of the malt-phosphate-bromate baking formula. It is evident that a wide

TABLE I

DESCRIPTION, PROTEIN, ASH AND BAKING DATA ON FLOURS USED IN THIS STUDY<sup>1</sup>

Sample No.	Type of flour	Crude protein (N×5.7)	Ash	Absorption	Loaf volume	Color	Texture
		%	%	%	cc.	%	%
1	Hard red spring	13.1	0.51	67	695	94.5	93.0
2	Hard red winter (Turkey)	12.3	0.51	58	616	93.5	92.5
3	Hard red winter (Chiefkan)	10.5	0.48	60	525	92.5	93.5
4	Soft red winter	8.5	0.48	58	469	92.5	94.0
5	Durum (Mindum)	17.1	0.88	63	465	90.0	93.0

<sup>1</sup> 13.5% moisture basis. The malt-phosphate-bromate formula was used in obtaining the baking data.

range of baking strength was included in the samples used in this investigation. These variations were, no doubt, due to differences in protein quality as well as protein quantity. The behavior of the durum sample is especially noticeable as this wheat had a high protein content, but showed poor baking performance, yielding a loaf of low volume and poor appearance. Chiefkan was substantially below the other hard wheats in loaf volume and crumb color.

In Table II are presented the viscosity data, expressed as centi-

TABLE II  
DENSITY, VISCOSITY, CONCENTRATION, AND FRACTIONATION DATA FROM SODIUM SALICYLATE DISPERSIONS OF GLUTEN WASHED FROM VARIOUS CLASSES OF WHEAT

Class of wheat	Wet gluten	Density	Viscosity	Dispersed protein per 100 cc.	Protein fractionated per 100 cc.
	%	<i>g. per cc.</i>	<i>centipoises</i> $\times 10^2$	<i>mg.</i>	<i>mg.</i>
Hard red spring	2.0	1.0401	155.7	470	198
	3.0	1.0411	169.3	698	304
	4.5	1.0417	192.5	1026	471
	6.0	1.0419	217.5	1361	720
	10.0	1.0430	295.9	2450	1195
	15.0	1.0446	416.5	3300	1767
	20.0	1.0459	594.1	4184	3215
Hard red winter (Turkey)	2.0	1.0408	154.9	469	193
	3.0	1.0410	164.9	653	301
	4.5	1.0416	190.5	978	499
	6.0	1.0419	215.3	1342	692
	10.0	1.0425	279.5	2091	1071
	15.0	1.0441	379.2	3153	1639
	20.0	1.0462	499.2	3956	3247
Hard red winter (Chiefkan)	2.0	1.0347	135.7	425	146
	3.0	1.0365	145.9	656	246
	4.5	1.0372	159.4	864	443
	6.0	1.0370	171.0	1035	587
	10.0	1.0388	221.2	1887	1091
	15.0	1.0401	286.5	2753	1810
	20.0	1.0420	381.3	3238	2437
Soft red winter	2.0	1.0404	151.6	462	158
	3.0	1.0408	157.8	607	237
	4.5	1.0414	176.7	926	418
	6.0	1.0418	198.7	1257	613
	10.0	1.0424	258.5	1986	944
	15.0	1.0438	337.9	2972	1391
	20.0	1.0457	469.6	4030	3013
Durum	2.0	1.0400	146.8	489	160
	3.0	1.0407	155.3	730	260
	4.5	1.0420	172.9	1105	448
	6.0	1.0422	186.4	1376	607
	10.0	1.0421	242.5	2248	885
	15.0	1.0453	336.0	3342	1491
	20.0	1.0462	412.6	4012	3175

poises  $\times 10^2$ , obtained upon the gluten dispersions of these flours without the application of pressure. The concentrations of dispersed protein as well as the fractionation results are also included. Figure 1 presents these values in graphic form. As the data are rather difficult to evaluate from the table, the discussion will be concerned with the figures. From this figure it is evident that the hard wheats have

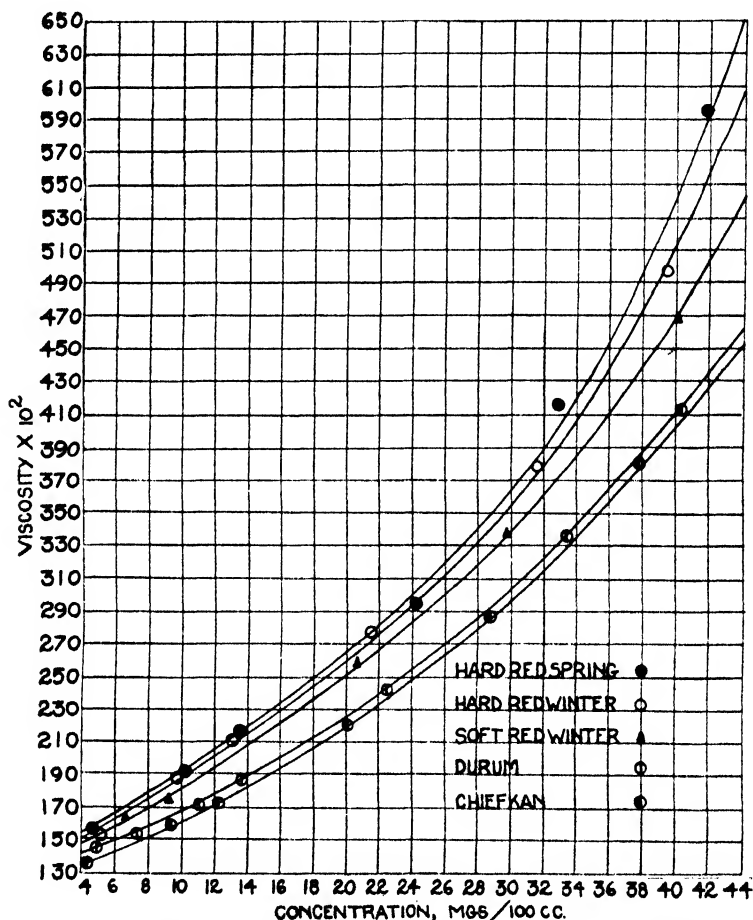


Fig. 1. Relationship between viscosity and gluten protein concentration. Concentration expressed as mg./100 per 100 cc. of dispersion.

higher viscosities than the soft winter or durum wheats throughout the range of protein concentration investigated. It is also evident that the viscosity increases more rapidly than concentration at the higher protein contents, principally because of the adsorption of the dispersion medium to form a hydrated shell around the protein micelle. It would appear that the particles from the stronger wheats were more highly hydrated than the weaker wheats, and this is in agreement with the usual concepts of the hydration capacities of strong and weak

flour gluten. It will be noticed that the hydration of the hard wheats increased more rapidly than that of the soft wheat, and the soft wheat in turn became hydrated more rapidly than durum or Chiefkan.

In Table III the results obtained by computing the hydrodynamic volumes<sup>1</sup> are shown, with the corresponding viscosities. For lyophilic colloids, the quantity  $\phi$  is much larger than the product of the con-

TABLE III

THE HYDRODYNAMIC VOLUMES OF PARTICLES OF GLUTEN WASHED FROM VARIOUS WHEATS AND DISPERSED IN 10% SODIUM SALICYLATE

Concentration—4000 mg. of protein per 100 cc.

Class of wheat	$\eta$ <sup>1</sup>	$\eta r$ <sup>2</sup>	$\phi$ <sup>3</sup>	Vol. occupied by 1 g. ( $\phi/C$ )
	<i>centipoises</i> $\times 10^2$	<i>centipoises</i> $\times 10^2$	%	cc.
Hard red spring	544	428.3	28.2	7.05
Hard red winter (Turkey)	570	401.6	27.3	6.82
Hard red winter (Chiefkan)	405	318.9	23.2	5.80
Soft red winter	468	368.5	25.8	6.45
Durum (Mindum)	408	321.3	23.5	5.87

<sup>1</sup> Values interpolated from Figure 1.

<sup>2</sup>  $\eta r = \eta/\eta_0$  where  $\eta_0$  = viscosity of dispersion medium (127).

<sup>3</sup> Values obtained by interpolation from graph prepared from data presented on page 53 of *Outlines of Biochemistry* by R. A. Gortner, 2nd ed., Chapman and Hall.

centration and the specific volume of the dispersed phase. Similarly, the quotient  $\phi/C$  is much larger than the specific volume. The larger volume may be due to the fact that the dispersed particles are either greatly hydrated or are interlocked and connected to form a micellar structure. As pointed out by McBain (1926), however, true hydration must be a factor in determining the swelling and aggregation and it accordingly appears that high viscosity must be related to the hydration of the dispersed protein particles. The mechanical immobilization of the dispersion medium by the presence of bulky aggregates or by elongated particles that increase the resistance to shear through mutual entanglement must, on the other hand, have a substantial effect upon viscosity. The electro-viscous effect is assumed to be of minor importance in this connection, although its effective magnitude is unknown. From an approximate determination of the migration

<sup>1</sup> These computations were based upon the equation of Kunitz:

$$\eta/\eta_0 = \frac{1 + 0.5\phi}{(1 - \phi)^4}$$

Where  $\eta$  = the coefficient of viscosity of the protein sol.  
 $\eta_0$  = the coefficient of viscosity of the dispersion medium.  
 $\phi$  = the percentage of the system occupied by the volume of the dispersed phase.  
 $C$  = concentration of the dispersed phase.

Kunitz, M. An empirical formula for the relation between viscosity of solution and volume of solute. *J. Gen. Physiol.* 9: 715-725, 1926.



of the dispersed colloid under an electric potential this effect appeared to be relatively small, as would be expected from the pH of the dispersions. The hydrodynamic volumes appear to range themselves in the same order as the "strengths" of the wheats.

The relationships depicted in Figure 2 indicate that plastic flow exists in 40% sucrose, 40% sodium salicylate, hard red spring, hard

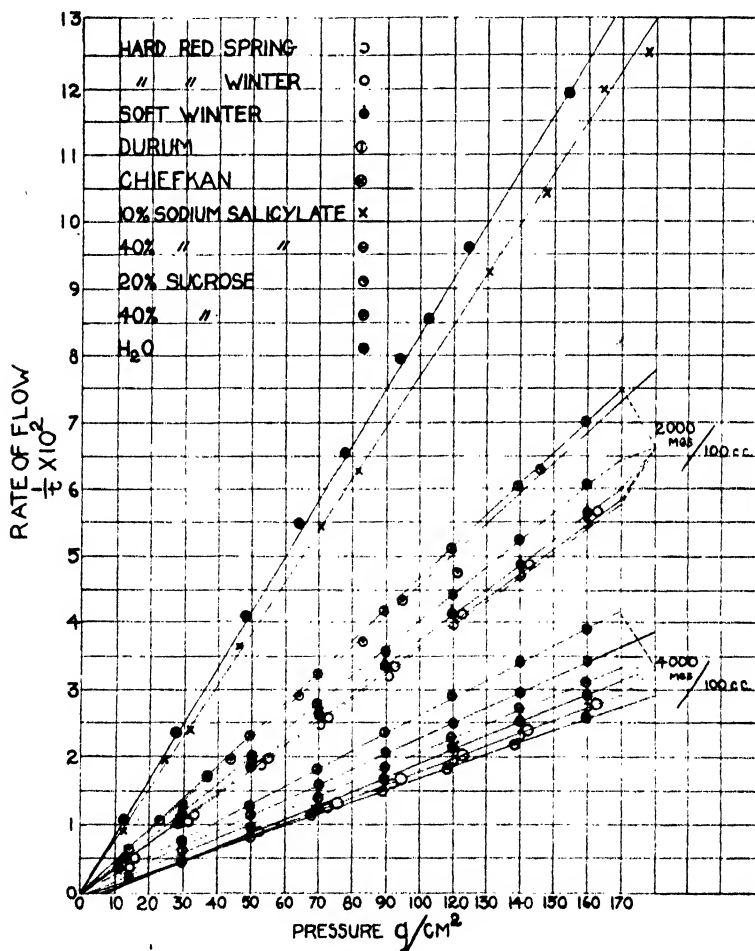


Fig. 2. Relationship between flow and pressure, time ( $t$ ) being expressed in seconds.

red winter (Turkey), and soft red winter gluten dispersions at concentrations of 4000 mg. of protein per 100 cc. True viscous flow evidently exists in the other liquids investigated, as the lines representing rate of flow with increments of pressure apparently pass through the origin, whereas in the instances mentioned above the lines passed to the right of the point of origin on the pressure axis. A certain amount of "yield" is thus indicated with the application of

pressure before flow actually starts. The plastic flow which apparently exists in the 40% concentrations of sucrose and sodium salicylate must be due to the relatively large number of molecules present. No plastic flow was found by Harris and Johnson in a previous investigation of the relationships between rate of flow and pressure, but the protein concentrations employed in that work were lower than in the present study. The formula used for calculating the total pressure employed in investigating effects of pressure upon viscosity was  $[(d_1)(h)] + [(\Delta p)d_2]$ , where  $d_1$  is the density of the liquid as determined by a standardized picnometer,  $h$  is the average height of liquid in the viscosimeter bulb and corresponds to the point mid-way between the upper and lower constrictions of the bulb,  $p$  is the pressure applied, expressed in cm. of mercury, and  $d_2$  is the density of mercury. Where pressure was used the viscosities were calculated from the equation

$$\eta = \frac{[(d_1)(h) + (\Delta p)d_2]t_2}{hd_3t_1}$$

where  $d_1$ ,  $h$ ,  $\Delta p$ , and  $d_2$  have the same values as before while  $d_3$  is the density of the reference liquid,  $t_2$  is the time of flow at the corresponding pressure, and  $t_1$  is the time of flow of the reference liquid. Time was expressed in seconds.

The data obtained with pressure were computed to a definite protein concentration by reading off the viscosity values for the desired concentration from the curves shown in Figure 1. This value was then recalculated in terms of flow,  $1/t \times 10^2$ , and located in Figure 2. By interpolation and suitable calculations employing a proportionality ratio, the corrected value was determined. The viscosity could then be found from the corrected flow-pressure diagram. The additive experimental error inherent in the viscosity-pressure determinations is eliminated by this method of computation.

In Figure 3 the relationships of viscosity to pressure are shown. Two concentrations of sucrose and sodium salicylate solutions were included, in addition to redistilled water and two concentrations of the gluten protein dispersions. Increasing the concentration of the solute or the dispersed phase increased the viscosity of all the liquids examined. Water and 10% sodium salicylate solutions yielded straight-line relationships between viscosity and applied pressure, while 40% sodium salicylate and 20% sucrose solutions showed a small initial decrease in viscosity with increase in pressure. When the sucrose concentration was raised to 40%, however, a marked curvilinear effect was obtained extending to approximately the 90 g./cm.<sup>2</sup> pressure region. It is thus apparent that this curve in the first por-

tion of the line is related, in the case of true solutions, to the concentration and probably also to the size of molecule, as the sugar molecule carries a hydrate layer which would increase its effective size. From

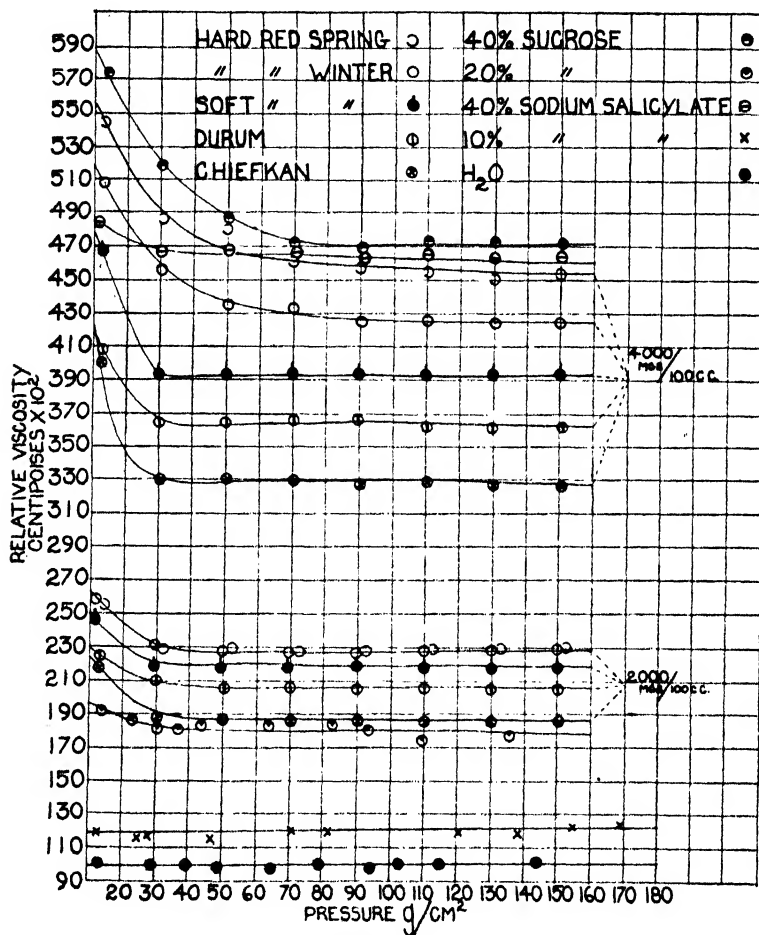


Fig. 3. Relationship between viscosity and pressure, as found in the different solutions and protein dispersions.

an examination of the curves for the gluten dispersions it appears that the same effect of concentration is found as in the instance of the solutions, that is, an increase in the degree of curvature with increase in concentration. This effect was present in the case of each dispersion irrespective of the class of wheat from which the gluten had been prepared, and is probably caused by surface tension, turbulent flow, and resistance at the capillary outflow as pointed out by the authors in a former paper (Harris and Johnson, 1940).

A table of correlation coefficients calculated from a fractionation and viscosity study conducted upon 30 samples of spring wheat flour

are shown in Table IV. A striking positive correlation was found between viscosity and mg. of protein removed from the dispersions by the addition of 6 cc. of concentrated  $\text{MgSO}_4$  solution to 100 cc. of the gluten protein dispersions. An even higher correlation between these variables was also obtained in the present study. It would therefore be possible to predict with fair accuracy the quantity of protein fractionated from the dispersions from a knowledge of viscosity. As the viscosity determination is the more accurate and precise of the two methods, is more rapid, and obviates the determination of nitrogen by the Kjeldahl procedure, it would appear that viscosity rather than fractionation is the more useful determination. No relationship of any practical significance was found between fractionation or viscosity and baking strength.

TABLE IV

CORRELATION COEFFICIENTS COMPUTED FROM STUDIES CONDUCTED ON FLOURS  
MILLED FROM NORTH DAKOTA HARD RED SPRING  
WHEAT—1938 CROP ( $N=30$ )

Variables correlated		Correlation coefficient $r_{xy}$	Proba- bility $P$
$X$	$Y$		
Protein fractionated (mg.)	Viscosity (centipoises)	<b>+ .8392</b> <sup>1</sup>	<.0001
Protein fractionated (mg.)	Flour protein (%)	-.0753	>.5530
Protein fractionated (mg.)	Loaf volume (standard) cc.	+.1731	.3453
Protein fractionated (mg.)	Loaf volume (M-P-B) cc. <sup>2</sup>	+.0023	>.5530
Density (g. per cc.)	Flour protein (%)	-.2131	.2436
Flour protein (%)	Loaf volume (M-P-B) cc.	<b>+.8664</b>	<.0001
Viscosity (centipoises)	Loaf volume (standard) cc.	+.1373	.4538
Viscosity (centipoises)	Loaf volume (M-P-B) cc.	+.0835	>.5530
FROM DATA IN PRESENT STUDY ( $N = 35$ )			
Protein fractionated (mg.)	Viscosity (centipoises)	<b>+.9592</b>	<.0001

<sup>1</sup> Significant values are printed in heavier type.

<sup>2</sup> M-P-B refers to malt-phosphate-bromate baking method.

### Summary and Conclusions

A series of gluten dispersions in sodium salicylate was prepared from hard red spring, hard red winter, soft red winter, and durum wheat flours. The effect of protein concentration upon viscosity was determined, as well as the rate of flow in relation to the pressure applied to force the liquid through an Ostwald pipette. The relationship between viscosity and pressure was likewise investigated. The concentrations used varied roughly from 4 to 42 mg. of protein per 100 cc. and the pressure from approximately 12.69 to 160 g. cm. of mercury. The protein concentration of the dispersions was ascertained by the Kjeldahl-Gunning method and the results obtained computed to a definite protein concentration basis.

The results indicated that the size of particle present in these dispersions varied in order of magnitude from hard red spring wheat through Turkey, soft red winter, and durum to Chiefkan. The hydration capacity apparently varied in the same order, which is very close to the general order of water absorption of these respective wheats and is in agreement with the general conception of their baking strengths. Redistilled water, 40% and 20% sucrose, and 40% and 10% sodium salicylate were also investigated. The gluten protein concentrations of dispersions used in the same series of experiments were 2,000 and 4,000 mg. per 100 cc. Large differences in rate of flow were found among these liquids. Redistilled water had the highest and 40% sucrose the lowest rate. Plastic flow was present to some extent in the 40% sucrose and 40% sodium salicylate solution, and in the hard and soft wheat dispersions at the higher concentrations. The durum and Chiefkan dispersions at this concentration were apparently truly viscous, but possibly would show plastic flow at higher concentrations.

An initial inflection in the curve representing viscosity and pressure relationships was obtained in every instance with the exception of redistilled water and 10% sodium salicylate. The degree of this curvature apparently increased with concentration and seemed to indicate a decrease in viscosity with increase in pressure in the lower pressure region. There was some indication that the harder wheats showed a more gradual change at the higher concentrations employed than did the other wheats examined.

The conclusions of Gortner and Doherty (1918) and Sharp and Gortner (1923) regarding differences in the physico-chemical properties of glutens prepared from strong and weak flours, and the relationship of these differences to the colloidal state of the gluten proteins, are confirmed by results obtained in this study. A statement of Rose and Cook (1935) regarding a possible correlation between viscosity values and baking quality is also substantiated to some extent when types of wheat varying widely in "strength" are considered. No significant relationship between these variables existed apparently among the samples of hard red spring wheat examined.

#### Acknowledgment

The authors wish to acknowledge the assistance of John Monge in preparing the figures and assembling the data contained in this paper.

#### Literature Cited

- Cook, W. H., and Alsberg, C. L.  
1931 Preparation of glutenin in urea solutions. *Can. J. Research* 5: 355-374.  
Cook, W. H., and Rose, R. C.  
1934 Solubility of gluten. *Nature* 134: 380-381.

- Gortner, R. A., and Doherty, E. H.  
1918 Hydration capacity of gluten from "strong" and "weak" flours. *J. Agr. Res.* **13**: 389-418.
- Harris, R. H.  
1937 A study of gluten protein fractionation from sodium salicylate solution. Part I. Durum wheat gluten fractionation. *Cereal Chem.* **14**: 695-707.  
1938 A study of gluten protein fractionation from sodium salicylate solution. Part II. Bread wheat gluten fractionation. *Cereal Chem.* **15**: 80-90.  
1939 A study of gluten protein fractionation from sodium salicylate solution. Part III. The effect of proteolytic enzymes. *Cereal Chem.* **16**: 78-88.
- and Johnson, John, Jr.  
1939 A study of gluten protein fractionation from sodium salicylate solution. Part IV. Effect of proteolytic enzymes, as influenced by class of wheat. *Cereal Chem.* **16**: 279-289.  
1940 A study of the effects of proteolytic enzymes and  $\text{KBrO}_3$  upon the viscosity and allied properties of dispersions of hard red spring wheat gluten. *Cereal Chem.* **17**: 203-222.
- McBain, J. W.  
1926 *J. Phys. Chem.* **30**: 239-247.
- Rose, R. C., and Cook, W. H.  
1935 Viscosity of gluten dispersed in alkali, acid and neutral solvents. *Can. J. Research* **12**: 63-81.
- Sharp, P. F., and Gortner, R. A.  
1923 Viscosity as a measure of hydration capacity of wheat flour and its relation to baking strength. *Minn. Agr. Exp. Sta. Tech. Bul.* **19**.

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## A SULPHUR-BEARING CONSTITUENT OF THE PETROLEUM ETHER EXTRACT OF WHEAT FLOUR (PRELIMINARY REPORT)<sup>1</sup>

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(Received for publication February 13, 1940)

It is well known that the baking quality of wheat flour is improved after being extracted by a fat solvent, such as petroleum ether. If the soluble material is recovered and added again to the extracted flour, the bread made from the mixture is usually much poorer than that from either the original or the extracted flour. The emphasis placed by Blish (1936) on the importance of flour lipoids and their possible protein combinations with respect to the quality of wheat bread led us to re-examine petroleum ether extract obtained from fresh patent flour.

An emulsion of the crude lipoidal extract in water was found to give a positive nitroprusside test. This property was lost after exposure to the air, but could be restored thereafter by adding alkaline cyanide. A partial purification of the constituent responsible for this reaction was then attempted. The test was made by mixing a drop of emulsion

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<sup>1</sup> Food Research Division Contribution No. 485.

with a drop of 2 *M* KCN on a spot plate. After two minutes a few milligrams of a powdered mixture of sodium nitroprusside and sodium carbonate (1-5) were stirred in.

A petroleum ether extract of flour was concentrated at room temperature and chilled, thereby removing considerable crystalline material that was completely negative when tested for sulfhydryl after a second crystallization.

The evaporated mother liquors could be freed from most of the fat, steroids, and pigments by solution in ether and dialysis against ether through a rubber membrane. The substance sought for was precipitated from the dialyzed ether solution by the addition of four volumes of acetone in the cold.

A method of purification was also devised to avoid the use of acetone and the possible reaction between it and a sulfhydryl group. Instead of dialyzing the ether solution it was mixed with three volumes of 1 *N* HCl in ethyl alcohol, whereupon a precipitate formed. After several hours in the cold, the precipitate was washed three times with absolute alcohol. Thereafter the reactive substance was no longer soluble in ether, so the precipitate was suspended in a large volume of ether for several hours. The material remaining undissolved was collected, dried *in vacuo*, and used for the tests reported here. It appeared to be more concentrated and more stable than that precipitated by acetone. About 0.33 g. was obtained per kilo of flour.<sup>2</sup>

The purified material was insoluble in all the ordinary organic solvents, but soluble in water and in 70% alcohol. The water solution gave a powerful nitroprusside test, but only after reduction with alkaline cyanide. Molisch's test and the biuret reaction were also positive. The solution turned black when boiled with an alkaline lead solution, presumably because of formation of lead sulphide.

On analysis the dried material was found to contain 13.4% of total nitrogen. Protein nitrogen by the trichloroacetic acid method (Northrup, 1932) was, however, only 3.9%, and the precipitate formed was gelatinous and unlike that usually obtained with proteins. Sulphur was found to be 2.94%, and phosphorus was absent in a test reliable to 1 part in 2000. When the solution was about two-thirds saturated with ammonium sulphate a precipitate formed that contained the reactive material.

It seems probable that the extractable flour lipoids contain a substance bearing a reversibly oxidizable SH group. The substance is no typical protein, but in some respects resembles a protein derivative. A question of obvious importance is whether it exists originally in

<sup>2</sup> The flour used was freshly milled unbleached patent flour from soft winter wheat. It contained 13.2% moisture, 8.15% protein, and 0.44% ash. We wish to thank the Wilkins-Rogers Milling Co., Inc., of Washington, D. C., for collecting this unbleached flour from the mill stream.

combination with a lipid or is merely soluble in lipid-containing fat solvents. This involves the further possibility that the purified material has been chemically changed during its separation from the lipoids. No answer can be given at this time.

The existence of a papain-like enzyme in flour and its influence on the bread-making qualities of the flour are now well known (Balls and Hale, 1938; Jørgensen, 1936). It is possible that the sulfhydryl-containing substance described here may serve as the natural activator of such a proteolytic system, and in this function be capable of modifying the gluten to a marked extent. As yet we have not been able to demonstrate the existence of this substance in commercially bleached flour. If one is willing to assume further that in the original flour the enzyme and such an activator are not always in intimate contact, then the unfavorable influence of added flour lipoids on the quality of the bread is easily explained. In any event we plan to investigate this interesting and reactive material further.

#### Literature Cited

- Balls, A. K., and Hale, W. S.  
1938 The preparation and properties of wheat proteinase. *Cereal Chem.* **15**: 622-628.
- Blish, M. J.  
1936 The wheat flour proteins. The Osborne Medal Award Supplement, *Cereal Chem.* **13**: 16-23.
- Jørgensen, H.  
1936 On the existence of powerful but latent proteolytic enzyme in wheat flour. *Cereal Chem.* **13**: 346-355.
- Northrup, J. H.  
1932 Crystalline pepsin. II. Hydrolysis and inactivation by acid. *J. Gen. Physiol.* **16**: 33-40.

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## REPORT OF THE 1938-39 SUBCOMMITTEE ON METHODS OF TESTING SELF-RISING FLOURS

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The Quaker Oats Company, St. Joseph, Missouri

(Read at the Annual Meeting, May 1939)

Collaborative baking and scoring data were collected on four samples of self-rising flour. Specific directions for baking procedure and scoring were followed. Definitions as given by McKim and Moss (1939) were used for evaluating the scores.

#### Collaborative Tests

Samples of self-rising flour were sent to each of the collaborators by the chairman. The identity of the flours was kept secret. Definite color scores were specified for flours No. 1 and No. 3, to be used as a



basis for judging the other two flours. The four samples used were the following:

No. 1—A soft wheat family patent, blended from three commercial samples of similar protein and ash.

No. 2—A soft wheat family patent having 0.6% higher protein and color similar to No. 1. (This was a blend of two commercial samples of similar protein.)

No. 3 and No. 4—The same hard wheat cut straight.

The formulae used were: For flours No. 1, 2, and 3, the self-rising formula as given by Walter (1936), (227.7 g. flour, basis 15.0% moisture; 3.4 g. soda; 4.3 g. hydrated mono-calcium phosphate; 4.6 g. salt); and for flour No. 4, additional phosphate over the above formula (227.3 g. flour, basis 15.0% moisture; 3.4 g. soda; 4.67 g. hydrated mono-calcium phosphate; 4.6 g. salt).

With this selection of samples and by using the same procedure and scoring, it was hoped to bring out the following points:

1. The relative biscuit-baking qualities of two soft wheat family patent flours having 0.6% difference in protein.
2. The effect of using excess phosphate on a hard wheat self-rising flour.
3. The ability of several collaborators in various localities to check on the scoring and rating of four *unknown* self-rising flours.

The results are summarized below in Table I.

Analyses, viscosity, and bread-baking tests on the plain flours were made by E. G. Bayfield. As a matter of record, these data are given (by permission) in Table II.

With these data in mind, answers to the three points mentioned above seem to be brought out as follows:

*First.* The biscuit-baking qualities of the two soft wheat family flours, which differed by 0.6% in protein, were similar, the collaborators scoring them close together in all respects. In Table II it is recorded that both the pH and the viscosity for flour No. 2 were lower than for No. 1. These properties of flour No. 2 may explain why the additional 0.6% of protein over that in flour No. 1 did not make any significant difference in the biscuits. On the other hand, biscuit-baking tests probably are not sufficiently sensitive to detect these comparatively small differences in flours.

*Second.* The effect of additional phosphate in the self-rising formula of the hard wheat flour did not materially improve the total biscuit score. Two of the collaborators found the color improved one

TABLE I  
REPORT OF COLLABORATIVE BAKINGS ON FOUR SELF-RISING FLOURS

	Collaborators					
	A	B	C	D	E	Av.
SAMPLE 1						
pH of biscuit	7.25	7.2	7.2	7.24	7.25	7.23
Oven loss %	16.4	11.3	16.5	8.5	12.2	13.0
Specific volume <sup>1</sup>	2.22	1.70	1.85	1.55	2.01	1.87
Total score	104.4	94	94.2	90	99.6	96.4
SAMPLE 2						
pH of biscuit	7.15	7.15	7.15	7.20	7.20	7.17
Oven loss %	15.0	10.9	16.7	8.3	13.2	12.8
Specific volume <sup>1</sup>	2.13	1.75	1.94	1.55	1.89	1.85
Total score	103.6	95	99	89	98	96.9
SAMPLE 3						
pH of biscuit	7.35	6.75 <sup>2</sup>	7.25	7.41	7.40	7.35
Oven loss %	13.9	10.8	15.1	7.1	12.3	11.8
Specific volume <sup>1</sup>	2.01	2.00	1.78	1.48	1.95	1.84
Total score	89.1	95	78.5	78	89.7	86
SAMPLE 4						
pH of biscuit	7.02	6.65 <sup>2</sup>	7.15	7.08	7.05	7.08
Oven loss %	14.9	10.9	16.2	9.2	12.1	12.7
Specific volume <sup>1</sup>	2.09	1.90	1.78	1.45	1.92	1.83
Total score	91.8	88.0	84.5	77.0	89.8	86.2

<sup>1</sup> Basis weight of dough.<sup>2</sup> Omitted from average.

TABLE II  
REPORT ON THE PLAIN FLOURS USED BY 1938-39 SELF-RISING FLOUR COMMITTEE

Sample	Flour					Viscosity		
	Protein <sup>1</sup>	Ash <sup>1</sup>	Moisture	Absorption <sup>1</sup>	pH 30°	20 g. flour	2 g. protein	No time
	%	%	%	%		° MacM.	° MacM.	° MacM.
No. 1	8.1	.347	9.5	51.9	5.40	70	119	59
No. 2	8.7	.327	10.0	53.2	5.10	63	89	72
No. 3	10.5	.471	9.6	60.0	5.76	142	117	92
Sample	Standard bake				Malt-phosphate-bromate bake			
	Loaf vol.	Grain	Texture	Crumb color score	Loaf vol.	Grain	Texture	Crumb color score
	cc.				cc.			
No. 1	552	98	87	100w	520	90	87	100w
No. 2	513	100	94	100w	477	92	91	100w
No. 3	655	98	100	93cw	676	100	100	91cw

<sup>1</sup> Basis 15.0% moisture.

point with the additional phosphate. The pH of the biscuits, as would be expected, was lowered 0.27 of a point.

*Third.* The total scores assigned to the biscuits did not check very closely. Differences in volume, which are evaluated by actual measurement, were the chief cause of variation between collaborators. The other items on the score sheet, which are evaluated mostly by personal judgment, checked rather closely in most cases. All collaborators agreed quite well in ranking Nos. 1 and 2 close together. They did not agree so well in ranking Nos. 3 and 4. This may be due in part to the fact that two or three of the collaborators were not accustomed to handling hard wheat flour biscuit doughs. Volume differences mentioned above can be caused both by manipulation of the biscuit dough and by the ovens. With the exception of two results by Collaborator B, the pH results on the baked biscuits checked very closely. Part of these pH values were determined colorimetrically and part with the electric pH meter. Oven losses varied considerably between collaborators but averaged out very nearly the same for all four flours. It is interesting to note that the *average specific volumes* of the four samples varied only 0.04 (from 1.87 to 1.83) in spite of the wide differences in the flours.

In Bayfield's data (Table II) the similarity in viscosity of soft wheat flour No. 1 and hard wheat flour No. 3, when based on 2 g. of protein, is quite possibly related to the higher ash content of flour No. 3. In his bread-baking data the crumb-color score and the loaf-volume score seem to correlate with biscuit scores, the color directly and the volume inversely, a large volume suggesting too much strength for best biscuit production when this test formula is used.

### Baking Procedure and Score Sheet

The baking procedure recommended follows that reported by Walter (1935) except for the incorporation of the one-fold, two-roll method recommended by the 1937-38 committee, and a slight change in mixing with the Hobart-type mixer, from 10 seconds to 15 seconds after the addition of the liquid.

The values assigned the different items in the score sheet have been retained. For purposes of simplification, it is suggested that the two items "Flour Quality" and "Eating Quality," heretofore listed under "Flavor" for a score of ten points each, be consolidated as one item, "Flavor," valued at 20 points. Under the "Remarks" column anything unusual about the flour or biscuits should be noted. In scoring, the definitions and methods of evaluating given by McKim and Moss (1939) should be followed. In judging color, the use of the

permanent biscuit standards reported by Percy and Putnam (1939) may be applied.

It is the consensus of opinion of this committee that since the *biscuit-baking test* is not critical in determining ordinary *variations in flour quality*, it should be considered a test for determining the following properties of a flour or of a self-rising flour mixture: (1) flour soundness and flavor, (2) evaluation of color, (3) proper chemical balance of self-rising ingredients, and (4) leavening power of the self-rising ingredients.

### Recommendations

It is the recommendation of this committee that these methods be divorced from soft-wheat testing, and that the program be made more general in character to include all types of both hard and soft wheat flour that may be made into chemically leavened products. It is suggested that the succeeding committee investigate:

1. The possibility of baking to a uniform oven loss as a means of bringing collaborators to a common basis which may result in more uniform biscuit volumes; and

2. The use of standard flours to smooth out personal, machine, and atmospheric differences in performing the biscuit-baking test.

### Acknowledgments

Members of this year's committee were: R. A. Barackman, H. V. Moss, Elizabeth McKim, Harold McGhee, C. C. Walker, Elmer Modeer, and O. E. Gookins. Acknowledgment is given for the able assistance of Jay Hedding in Mr. McGhee's laboratory and Claribel Albright in the Quaker Oats Company laboratory.

### Literature Cited

Gookins, O. E.

1939 Report of the 1937-38 subcommittee on methods of testing self-rising flours. *Cereal Chem.* **16**: 113-116.

McKim, Elizabeth, and Moss, H. V.

1939 Study of definition and evaluation of various items on score card. *Cereal Chem.* **16**: 117-136.

Pearcy, G. W., and Putnam, H. W.

1939 Convenient crumb color standards for self-rising flours. *Cereal Chem.* **16**: 127-130.

Walter, H. G.

1935 Report of the 1933-34 committee on methods of testing self-rising flours. *Cereal Chem.* **12**: 162-164.

1936 Report of the 1934-35 committee on methods of testing self-rising flours. *Cereal Chem.* **13**: 722-723.

## 1938-1939 REPORT OF THE SUBCOMMITTEE ON TESTING BISCUIT AND CRACKER FLOURS

HOWARD M. SIMMONS, *Chairman*

The Mid-West Laboratories Co., Inc., Columbus, Ohio

(Read at the Annual Meeting, May 1939)

The program followed by the 1938-1939 subcommittee was planned to deal with two of the recommendations made by the previous committee (Brown, 1939), namely: to determine whether there is a correlation between the classification of flours made by the laboratory baking test and that made by a viscosity test after the addition of 1 cc. of lactic acid (as suggested by Triebold), and whether further correlations are possible between laboratory evaluations of cracker flours and shop performance. No work was attempted on cooky flours.

The committee used the same procedure followed by previous committees. In order to secure additional data, four Pacific Coast cracker flours were studied in addition to four from the Middle West. Members of previous committees have agreed satisfactorily on the chemical analyses of the flours used; hence analyses of these flours were made in one Pacific Coast and one Middle Western laboratory respectively.

One member made all the bread-baking tests and sent a loaf from each flour sample to each member of the committee for scoring and classifying for use as sponge and/or dough flour.

Viscosity tests were made following the method outlined by Bayfield (1936), procedures No. 1 and No. 2. On the basis of his own viscosity results and the analytical data furnished with the samples, each committee member classified the flours.

Crackers were baked in commercial shops following schemes used by Reiman (1938) and Brown (1939); *i.e.*, the two sponge flours were baked respectively with the two dough flours from the same district. Because of circumstances beyond control of the committee the proportions used in the bakings on the Pacific Coast were altered. Four barrels of sponge flour No. 9 were used respectively with dough flours No. 11 and 12, while only  $3\frac{1}{2}$  barrels of sponge flour No. 10 were used with  $1\frac{1}{2}$  barrels of Nos. 11 and 12. These differences may have tended to equalize the results. Two bakings were made on the Pacific Coast flours; three on the Middle Western. Each collaborator scored and evaluated the resulting crackers according to the method outlined in Cereal Chemistry 15: 37. One member analyzed the baked crackers and another determined the shortometer values.

\* For the sake of continuity, the flours were designated as Nos. 9, 10, 11, 12, 13, 14, 15, and 16. Numbers 9 to 12 were Pacific Coast flours,

while 13 to 16 were from the Midwest. Table I shows the analytical data and the type of wheat from which each flour was milled. These flours were selected for the work this year on the basis of their analyses and viscosities.

TABLE I  
ANALYTICAL RESULTS OF THE FLOUR STUDIED

Sample number	Ash at 15% H <sub>2</sub> O	Protein at 15% H <sub>2</sub> O	pH value	Used for	Wheat used
9	.403	8.98	5.75	Med. Sponge	Baart & Soft White
10	.416	10.26	5.82	Strong Sponge	Baart & Soft White
11	.372	7.35	5.47	Weak Dough	Soft White
12	.381	7.50	5.73	Strong Dough	Soft White
13	.355	8.40	5.82	Strong Sponge	Red
14	.385	8.28	6.00	Med. Sponge	Red
15	.368	7.83	6.25	Strong Dough	Red
16	.378	7.21	6.10	Weak Dough	Red & White

### Discussion of Results

The viscosity results obtained by the collaborators showed quite close agreement with the no-time method. There was a little greater spread among the results with the one-hour digestion method. This may have been due to the slightly greater sensitivity of the method. Table II gives the averages by both methods.

TABLE II  
AVERAGE VISCOSITY DETERMINATIONS BY ONE-HOUR AND NO-TIME METHODS

Sample No.	9		10		11		12	
	1 hr.	No time	1 hr.	No time	1 hr.	No time	1 hr.	No time
Average	91	64	105	78	47	33	61	41

Sample No.	13		14		15		16	
	1 hr.	No time	1 hr.	No time	1 hr.	No time	1 hr.	No time
Average	94	73	82	65	68	51	39	29

The results obtained after the addition of one cc. of lactic acid showed a very wide spread between collaborators. The committee members feel that because of this widespread disagreement some study should be made by the Viscosity Committee, or a Section Committee, to see if these results cannot be made to check as well as our final viscosity determinations.

Table III gives the classification of the flours by the collaborators based on the viscosity tests. These classifications are based on the

TABLE III  
CLASSIFICATION OF FLOURS ON BASIS OF ANALYTICAL AND VISCOSITY TESTS

Collaborator no.	Flour sample							
	P1 9	P2 10	P3 11	P4 12	M1 13	M2 14	M3 15	M4 16
ONE-HOUR METHOD								
1	Sponge	Sponge	Dough	Dough	Sponge	Sponge	Dough	Dough
2*	Strong sponge	Very st. sponge	Dough	Dough	Sponge	Sponge	Sponge or Dough	Dough
3	Sponge or Dough	Sponge or Dough	Cooky	Dough	Sponge	Sponge	Dough	Dough
4	Strong sponge	Very st. sponge	Dough	Strong dough	Sponge	Sponge	Weak sponge	Dough
5	Sponge	Strong sponge	Cooky	Cooky	Weak sponge	Dough	Cooky	Weak cooky
6	Sponge	Sponge	Cooky	Dough	Sponge	Sponge	Dough	Cooky
7	Sponge	Sponge	Dough	Dough	Sponge	Sponge	Dough	Dough
8	Strong sponge	Strong sponge	Weak dough	Weak dough	Sponge	Sponge	Strong dough	Weak dough
NO-TIME METHOD								
1	Sponge	Sponge	Dough	Dough	Sponge	Sponge	Dough	Dough
2*	Sponge	Sponge	Cooky	Dough	Sponge	Sponge	Sponge dough	Cooky
3	Sponge	Sponge	Cooky	Dough	Sponge	Sponge	Dough	Dough
4	Sponge	Sponge	Dough Cooky	Dough	Sponge	Sponge	Dough	Dough
5	Sponge	Strong sponge	Cooky	Cooky	Weak sponge	Dough	Cooky	Weak dough
6	Sponge	Sponge	Cooky	Dough	Sponge	Sponge	Dough	Cooky
7	Sponge	Sponge	Dough	Dough	Sponge	Sponge	Dough	Dough
8	Sponge	Sponge	Cooky	Dough	Sponge	Sponge	Strong dough	Cooky Wk. do.

\* Classification made from average viscosity tests.

final reading by both the one-hour and the no-time method. The results on the whole are in fairly good agreement by both methods.

If there is any difference, the one-hour method would place the borderline flours slightly higher than they would be placed by the no-time method. Flour No. 10 rates as a sponge flour by the no-time method, but as a very strong sponge by the one-hour method. One collaborator reports, "Almost too strong for a sponge flour."

Flour No. 15 was rated as a dough flour by most collaborators with both methods. However, in two cases it was rated a degree higher.

The classification of flours from the baking test, given in Table IV, on the whole shows close agreement and parallels the classification of the flours by the viscosity test, with the exception of No. 15, which was rated a dough flour by that method but classed as a sponge flour by the baking test. This would tend to confirm Reiman's (1938) observations that "wherever there was a difference in rating of a flour by the two methods, *i.e.*, the baking and viscosity test, the rating of the baking test was a degree higher than by the viscosity test." The classification of these eight flours also seems to confirm Reiman's observation, "that the collaborators were able to reach the same conclusions with

TABLE IV  
CLASSIFICATION OF FLOURS BY BAKING TEST

Collaborator no.	Flour sample							
	P1 9	P2 10	P3 11	P4 12	M1 13	M2 14	M3 15	M4 16
1	Sponge	Sponge	Dough	Dough	Sponge	Sponge	Weak sponge	Weak dough
2	Dough or Sponge	Strong sponge	Cooky	Dough	Dough or Sponge	Sponge	Sponge	Cooky
3	Sponge or Dough	Sponge or Dough	Dough	Dough	Sponge	Sponge	Sponge	Dough
4	Sponge	Sponge	Dough	Dough	Strong sponge	Sponge	Sponge	Cooky
5	Sponge	Sponge	Cooky	Cooky	Sponge	Sponge	Sponge	Cooky
6	Sponge	Sponge	Dough	Dough	Sponge	Sponge	Sponge	Cooky
7	Sponge	Sponge	Dough	Dough	Sponge	Sponge	Sponge	Dough or Cooky
8	Sponge	Strong sponge	Weak dough	Dough	Sponge	Sponge	Weak sponge	Dough or Cooky

respect to the flours from loaves baked by someone else as from their own loaves." The results for the cracker scores show the same tendencies as those obtained by the two preceding committees; *i.e.*, "There is a considerable difference between the way individuals score crackers both in valuation and their rating."

Table V shows the rankings of the crackers baked from Pacific Coast flours. The total scores on the two bakes agree only on the No. 4 cracker, which was rated No. 4 on both bakes. On the composite rating of the two bakes, the No. 3 cracker rates third and the No. 4 cracker rates fourth. These crackers were baked with the No. 10

TABLE V  
RANKING OF P CRACKERS BY SCORES AND SHORTOMETER VALUES

Collaborator no.	First bake—Sample				Second bake—Sample			
	P1	P2	P3	P4	P1	P2	P3	P4
1	4	3	2	1	3	2	1	4
2	3	4	2	1	4	1	3	2
3	2	1	4	2	2	4	1	4
4	1	1	4	2	4	1	2	2
5	2	3	1	4	2	2	1	4
6	1	2	4	3	1	2	4	3
7	3	2	1	4	2	2	1	4
8	1	3	2	4	3	2	4	1
Total	17	19	20	21	21	16	17	24
Rank	1	2	3	4	3	1	2	4

Cracker	SUMMARY				SHORTOMETER RANK			
	P1	P2	P3	P4	P1	P2	P3	P4
First Bake	1	2	3	4	2	1	3	4
Second Bake	3	1	2	4	4	1	3	2
Total	4	3	5	8	6	2	6	6
Rank	2	1	3	4	2	1	2	2



flour as a sponge and rated by both baking test and viscosity test as "sponge" to "very strong sponge," with most collaborators rating it as a strong or very strong sponge by the one-hour digestion method. This flour is probably approaching the upper limits as a sponge flour, and it appears, in this instance, that viscosity by the one-hour digestion method more properly classifies this flour than the no-time method. This would tend to confirm Reiman's (1938) and Brown's (1939) observations, that "the quality of crackers seems to be more dependent upon the type of sponge flour than the type of dough flour used in the formulae."

The shortometer values on the first bake follow the ranking of the crackers, but on the second bake they were not so close. The No. 2 cracker rates the highest by the shortometer and by the cracker scores.

Table VI shows the ranking of the crackers baked from the Midwest flours. The total rankings on the first two bakes were exactly the same. However, in the third bake this was true only for *Cracker C*. As one member of the Committee states, "With all the crackers being first class, it is harder to pick the winner than the loser."

TABLE VI  
RANKING OF M CRACKERS BY SCORES AND SHORTOMETER VALUES

Collabo- rator no.	First bake—Sample				Second bake—Sample				Third bake—Sample			
	A	B	C	D	H	G	F	E	M	N	O	P
1	3	1	2	4	1	1	4	2	1	1	4	3
2	1	3	4	2	3	2	4	1	2	4	3	1
3	4	2	2	1	2	2	1	4	4	2	3	1
4	1	1	4	4	1	3	4	2	3	1	4	2
5	1	4	3	2	2	1	3	4	2	1	4	3
6	4	1	2	3	3	2	4	1	3	2	4	1
7	4	1	1	2	1	2	3	4	4	3	1	2
8	1	3	4	2	2	1	4	3	3	4	2	1
Total	19	16	22	20	15	14	27	21	22	18	25	14
Rank	2	1	4	3	2	1	4	3	3	2	4	1

Cracker	SUMMARY				SHORTOMETER RANK			
	A	B	C	D	A	B	C	D
First Bake	2	1	4	3	2	1	4	3
Second Bake	2	1	4	3	3	2	4	1
Third Bake	3	2	4	1	3	4	2	1
Total	7	4	12	7	9	7	10	5
Rank	2	1	4	2	3	2	4	1

The composite ranking on all three bakes places *Cracker C* in last place and the *Cracker B* in first place, while *A* and *D* rate the same, although on the basis of the first two bakes the *A* cracker would rate second place and *D* third.

*Crackers A and B* were baked with the stronger sponge flour while *C and D* were baked with the weaker sponge flour. *Cracker C*, which rates last place, had the very strong borderline No. 15 flour used in the dough, as also did the *A* cracker, which rated second place. Here again the characteristics of the sponge flour No. 13 might be such that it was able to work with both dough flours better than the weaker No. 14 sponge flour.

Again the shortometer values followed the rankings of the cracker for the first bake and were not widely different for the next two bakes. *Cracker C* was rated last by both the collaborators' scores and the shortometer.

The chemical and physical analyses of the crackers (Table VII), while not differing greatly from those reported by the two preceding committees, do show some points of variation. The thickness of ten crackers is about the same as last year, but the count per pound is much lower. Last year, Brown had an average around 140 per pound on all three bakes. This year the count per pound was from 107 to 123, which is about the average found by Reiman's committee two

TABLE VII  
CHEMICAL AND PHYSICAL ANALYSIS OF CRACKERS

Bake No.	Cracker No.	Mois- ture as red'd	Protein N×6.25	Ash	Fat	Shorto- meter value	Av. pH	Av. thickness of ten	Av. count per lb.
1-P	1-P	5.08	9.58	2.46	11.58	86.6	8.0	2 12/16	122
	2-P	5.20	9.76	2.59	11.85	83.7	8.4	2 13/16	121
	3-P	5.63	10.19	2.17	11.56	87.3	8.0	2 11/16	123
	4-P	5.30	10.19	2.62	12.01	87.6	8.1	2 12/16	121
2-P	1-P	5.20	9.72	2.14	11.72	83.7	7.9	2 13/16	118
	2-P	4.60	9.63	3.17	11.75	67.5	8.2	2 13/16	123
	3-P	5.33	10.15	2.12	11.97	82.7	8.0	3	117
	4-P	4.18	10.41	2.70	11.86	71.9	7.1	2 15/16	120
3	A	4.80	8.75	2.72	13.87	76.0	8.1	2 13/16	115
	B	4.05	8.66	2.70	13.87	70.0	8.1	2 12/16	118
	C	5.25	8.62	2.53	13.34	90.0	8.1	2 13/16	109
	D	3.93	8.58	2.91	13.56	76.1	7.7	2 14/16	111
4	A	5.30	8.36	3.72	14.08	81.6	8.0	2 13/16	114
	B	4.65	8.18	4.14	14.18	80.2	7.8	2 12/16	115
	C	5.45	8.40	3.30	13.60	85.2	8.0	3 1/16	105
	D	5.15	8.09	4.38	13.91	76.0	7.45	2 13/16	107
5	A	4.60	8.76	2.55	13.63	76.6	7.8	2 15/16	108
	B	4.20	8.66	2.15	13.61	80.6	7.6	2 12/16	114
	C	4.60	8.62	2.55	13.47	72.4	8.0	2 14/16	113
	D	3.90	8.49	2.75	13.37	63.9	8.1	2 13/16	114

Chemical analysis by T. E. Hollingshead. Shortometer values by H. J. Loving.

years ago. The average fat content was lower last year than that found this year and two years ago. There seems to be some relationship between the moisture content of the cracker and the shortometer value. The higher the moisture, the higher the shortometer reading.

### Conclusions

The work done this year has been essentially a repetition of work done by previous committees. However, it is felt that the results serve as a substantial confirmation of the work of those committees on the evaluation of flours by the baking and viscosity tests.

We find these methods of evaluation hold equally as well with the Pacific Coast flours as with the flours previously studied.

Results seemed to indicate that the one-hour digestion viscosity method gave a sufficiently reliable classification of these flours to warrant further consideration.

This year the viscosity at the one-cc. lactic acid level was observed particularly, in accordance with the recommendation of the previous committee. Discussion of the results observed indicate that at the one-cc. level the system is not sufficiently stable to give a reliable picture.

### Recommendations

The Committee recommends:

That further work of this same type be done to build up a greater volume of data to correlate more closely the laboratory evaluation of cracker flours with their actual shop performance.

That further study be made on the scoring of crackers in order to obtain a closer agreement among collaborators.

That a statistical study be made of all pertinent data accumulated by the committees in the last three years; this to be done in order that more practical conclusions may be reached.

### Acknowledgments

To the members of this committee, C. C. Armuth, E. F. Evert, H. J. Loving, T. E. Hollingshead, H. O. Triebold, O. P. Skaer, and Miss Pearl Brown, the chairman wishes to express his appreciation and thanks for the hard work and loyal cooperation given.

The chairman also wishes to express his appreciation to the American Cracker Company of Seattle, Washington, whose general manager, R. P. Thymain, and production manager, A. G. Matsten, gave us full cooperation, and to the Kroger Grocery and Baking Co., their purchasing agent, H. H. Wirtz, and their superintendent of cracker bakeries, R. F. Lovell, and to W. S. Culver, who also gave us the best of cooperation.

Also, the chairman is personally indebted to Messrs. Garnatz and Putnam for the many helpful suggestions in planning this work, and to E. G. Bayfield and his staff for baking the bread.

## Literature Cited

- Bayfield, E. G.  
 1936 Report of subcommittee on viscosity. *Cereal Chem.* **13**: 773-788.  
 Brown, Pearl  
 1939 1937-1938 report of subcommittee on testing cracker and biscuit flours. *Cereal Chem.* **16**: 130-136.  
 Reiman, W.  
 1938 Report of 1936-1937 committee on testing biscuit and cracker flours. *Cereal Chem.* **15**: 35-48.

PRELIMINARY STUDY OF EIGHT TEST METHODS <sup>1</sup>

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(Read at the Annual Meeting, May 1939)

This report is a brief summary of the work done in studying eight methods for testing cake flours preliminary to the collaborative work of the 1938-39 Subcommittee on Methods of Testing Cake Flour. The eight methods, which included the official A.A.C.C. method, were submitted by committee members as being in use to some extent in their individual laboratories.

TABLE I

Form- ula No.	Flour	Short- ening	Sugar	Salt	Bak- ing pow- der	Milk	Eggs, whole or whites	Type of cake	Mixing method
1	100	37.0	120	3.7	5.5	70.4	56	White layer	Blending (3-stage)
2	100	44.5	111	2.2	4.4	89.0	40	White layer	Creaming
3	100	55.6	142	3.6	6.2	107.0	60	Yellow layer	Blending (3-stage)
4	100	38.0	120	3.2	4.8	80.0	60	White layer	Blending (3-stage)
5	100	57.2	100	2.8	2.1	51.0	64	Yellow loaf	Single stage
6	100	50.0	100	3.0	7.5	75.0	50	Yellow loaf	Single stage
7	100	54.6	140	3.8	5.0	96.0	75	White layer	Blending (3-stage)
8	100	25.0	96	1.5	5.8	88.0	32	White loaf	Single stage
(Official)									

The object of the work was chiefly: (1) to examine the methods for sufficiency of detail and experimental directions, and (2) to indicate if possible a formula or formulas which would be satisfactory for future work. This, it was hoped, would guide and expedite the later collaborative work.

## Cake Formulas

Table I identifies the test methods which were submitted by committee members. The first eight columns give the actual cake formulas on the common basis of flour as 100. The last two columns show

<sup>1</sup> Report of 1938-39 subcommittee on Methods of Testing Cake Flours.

the type of finished cake produced and the type of mixing employed by the test method.

### Flours

Nine samples of flour were submitted by the committee to be used in testing the eight formulas. The analyses<sup>2</sup> of the nine flours are shown in Table II.

TABLE II

	Flour samples								
	A	B	C	D	E	F	G	H	I
Protein (15% m. b.)	7.4	7.6	7.7	7.4	8.3	7.7	8.3	7.8	7.0
Ash (15% m. b.)	0.34	0.32	0.35	0.38	0.37	0.36	0.37	0.33	0.32
pH	5.2	5.1	5.1	5.0	5.0	5.1	5.0	5.1	5.0
Viscosity	46	57	59	27	55	56	52	57	42

### Experimental

Eight bakings of nine cakes each were made. Each baking of nine cakes represented one formula with the nine flours. On each of the 72 cakes the following data were obtained: baking loss, volume, symmetry, crust character, tenderness, silkiness of crumb, grain, color, batter temperature, batter pH, specific gravity, and baking temperature and time. Individual photographs of the cakes were made. All of these data, together with specific suggestions for improving details of the formulas, were submitted to the committee chairman.

### Discussion

The eight test units were chosen almost at random and varied widely, (1) in relative amounts of ingredients in the formula, (2) in mixing methods, and (3) in the type of cake to be baked (loaf or layer, yellow or white). In addition, the eight methods considered make no attempt to cover all the possibilities for a "best" test method. Thus the work is not broad enough in scope to permit the selection of a best method for differentiating between cake flours as to suitability. However, using this work in part as a guide, the Committee selected one of these test formulas to compare with the Official Method. The comparison was made in later collaborative work which is being reported separately.

<sup>2</sup> These analyses are the means of those obtained in the laboratories of J. W. Montzheimer, H. W. Putnam, and E. G. Bayfield.

## REPORT OF THE 1938-39 COMMITTEE ON METHODS OF TESTING SOFT WHEAT<sup>1</sup>

H. W. PUTNAM, *Chairman*

Igleheart Brothers, Incorporated, Evansville, Indiana

(Read at the Annual Meeting, May 1939)

The Committee on Methods of Testing Soft Wheat was set up in 1937 for the purpose of coördinating the activities of three committees of the Association then engaged in work on soft wheat flours. Cereal chemists and their associates in twenty-four laboratories as well as the employees of two commercial cracker bakeries contributed to the work accomplished by the committee in 1938-39.

Complex problems are involved in the proper classification and measurement of soft wheat flours. The ultimate use to which the products are to be put must be kept in mind constantly. Ideal methods of testing these special-purpose flours do not as yet exist. Baking tests must be used. These involve many variables and possibilities of error. To reduce these obstacles to a minimum and to learn how to test the soft flours under conditions which best will reveal their true quality in relation to the ultimate products in which they will be used have been the objectives even though not the achievements of this committee.

The sub-committee on Methods of Testing Cake Flour (J. W. Montzheimer, chairman) made further comparisons between the present test-cake formula and typical commercial-cake formulas. It seems probable that both the lean A.A.C.C. test-cake formula and a richer type may be required to properly classify cake flours. A valuable sub-project was executed by Messrs. Coughlin, Wade, and Green. Dr. Stamberg contributed to the standardization of the technique of scoring cakes for grain, symmetry, and volume and prepared photographs to help reduce differences between collaborators. More detailed directions need to be worked out for the procedure itself as well as for interpreting other characteristics of the cakes. In view of improved mixing equipment now available it is probable that specifications for mixing the test batters require reconsideration.

The Sub-committee on Methods of Testing Biscuit and Cracker Flours (Howard M. Simmons, chairman) carried out a project similar to that of its two predecessors but included Pacific Coast flours as well as midwestern flours. This committee has kept the laboratory evaluation of cracker flours closely correlated with shop usefulness. One of the chief problems is a dependable system for scoring the crackers.

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<sup>1</sup> General Report.

A thorough study is to be made next year of the data accumulated during the past three years in the hope of discovering trends not apparent before.

The Sub-committee on Methods of Testing Self-Rising Flours (O. E. Gookins, chairman) made some collaborative tests in order further to study scoring suggestions made last year. Whereas, for two years this committee has been part of the Committee on Methods of Testing Soft Wheat, it may be best to separate it since the biscuit baking test is not itself a critical flour test, but rather is a test which frequently is used for determining soundness in flour, flour color, and the balance between the chemical leavening ingredients regardless of whether the flour is soft or hard.

Methods for testing pie flours again were not considered this year.

Definite progress has been made by each sub-committee during the past two years. In view of the increasing interest in soft wheat problems, these co-operative and collaborative efforts should be continued. Definite recommendations concerning soft wheat testing should be prepared as an aid to the committee which may undertake revision of *Cereal Laboratory Methods*.

The author wishes to acknowledge the fine spirit of co-operation which has pervaded the work. Particular recognition and thanks are due the chairmen of the respective sub-committees.

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## ANNUAL REPORT OF TREASURER

OSCAR SKOVHOLT

January 1, 1940

The membership statement reveals a continuation of the Association's steady growth from the standpoint of numbers. The net increase of 30 members compares with a gain of 36 in the previous year.

The financial statements reveal that the surplus from the year's operations has exceeded that in any previous period. *Cereal Chemistry* income was appreciably increased over that in the previous year with a smaller increase in expenditures. A part of this income was due to a substantial surplus donated to the Association from the 1938 Cincinnati annual convention and assigned by the Executive Committee to the *Cereal Chemistry* fund. General Association expenditures were practically unchanged and there was a moderate increase in receipts.

During the year, the savings account in the Harris Trust and Savings Bank of Chicago was closed and this amount plus a sum from the checking account was invested in U. S. Savings Bonds. This action was authorized by the Executive Committee upon the advice of the Committee on Investments. These bonds, at a cost of \$6,000, have a maturity value of \$8,000 after ten years. The interest rate is 2.9% compounded semi-annually.

The account with the North American Savings and Loan Association of Missouri still consists of \$400 of class A stock, now with interest accrued to a total of \$30.89, and \$1,600 of class B certificates of undetermined value. Progress is reported in liquidating class B assets through the sale of property. Payments on these properties have not as yet been substantial enough to convert them to class A accounts. It

seems likely that the present book value as placed on this investment may be realized. This Savings and Loan Association has made application for, and has almost been assured of, the acceptance of its accounts by the Federal Savings and Loan Insurance Corporation.

Some of the details of the Treasurer's and Secretary's offices have recently been assumed by an employee of the Association in the office of the Managing Editor. This centralizes the matter of Association membership lists and eliminates considerable duplication of effort.

Sales of *Cereal Laboratory Methods* continue at an essentially unchanged rate with 80 copies being sold during 1939. Only 60 copies of the third edition remain at the end of the year.

#### DETAILED MEMBERSHIP STATEMENT DECEMBER 31, 1939

	Total	Active	Corp- oration	Honorary
Membership December 31, 1938.....	600	545	53	2
New members added during 1939.....	54	49	5	0
Members reinstated during 1939.....	7	7	0	0
Members resigned and suspended 1939....	30	26	4	0
Members lost by death.....	1	1	0	0
Members in good standing, Dec. 31, 1939...	630	574	54	2
Net increase in membership, 1939.....	30	29	1	0

#### PROFIT AND LOSS STATEMENT

January 1 to December 31, 1939

##### RECEIPTS

##### *Cereal Chemistry*

##### Membership Dues

Active.....	\$2,016.00
Sustaining.....	540.00
Subscriptions, reprints, back issues and advertising.....	7,057.01
1939 accounts receivable.....	284.14
Interest on invested funds.....	110.35
Cincinnati convention surplus.....	339.63
Appropriated by Association.....	200.00

Gross receipts.....	\$10,547.13
Less 1938 income received, 1939.....	326.79

Net receipts, 1939..... \$10,220.34

##### Association

Membership dues.....	2,009.00
Application fees.....	147.00
Interest on invested funds.....	106.60
Miscellaneous income.....	1.75

Net receipts, 1939..... 2,264.35

<i>Cereal Laboratory Methods</i> sales, 1939.....	227.45
Interest on invested funds.....	17.13

Net receipts, 1939..... 244.58

##### Decennial Index

Received from <i>Cereal Chemistry</i> .....	75.00
Received from Association.....	75.00

Net receipts, 1939..... 150.00

**TOTAL RECEIPTS OF ALL ACCOUNTS, 1939..... \$12,879.27**



## DISBURSEMENTS

*Cereal Chemistry*

Cost of printing journal and reprints.....	\$6,190.84	
Net cost of printing.....		\$ 6,190.84
Cost of editors and miscellaneous services....	2,055.68	
Less 1938 account paid 1939.....	6.34	
	<hr/>	
Net cost of editing.....		2,049.34
Decennial Index— <i>Cereal Chemistry</i> assessment		75.00
	<hr/>	
Disbursements 1939.....		8,315.18
1938 account—uncollectible.....		6.46
	<hr/>	
Total disbursements 1939.....		\$ 8,321.64
Net surplus 1939.....		\$1,898.70

## Association

Expenses of President's and Vice President's offices and <i>News Letter</i> .....	339.34	
Expenses of Secretary's office.....	218.08	
Expenses of Treasurer's office.....	136.05	
Committee expenses.....	84.52	
Convention ads in <i>Cereal Chemistry</i> .....	20.00	
Kansas City convention report.....	313.05	
Expenses of officers' section visits.....	69.45	
Appropriated to <i>Cereal Chemistry</i> .....	200.00	
Decennial Index—Association's assessment...	75.00	
Miscellaneous expense.....	12.10	
	<hr/>	
Net disbursements 1939.....		1,467.59
Surplus 1939.....		796.76

*Cereal Laboratory Methods*

Binding and mailing expenses.....	68.11	
Surplus 1939.....		176.47
Decennial Index		150.00
Surplus 1939.....		

TOTAL DISBURSEMENTS OF ALL ACCOUNTS.....	\$ 9,857.34	
	<hr/>	
TOTAL SURPLUS.....		\$ 3,021.93

## DISTRIBUTION OF NET ASSETS

<i>Cereal Chemistry</i> assets, 1938.....	\$ 5,310.46	
Surplus, 1939.....	1,898.70	
	<hr/>	
Assets December 31, 1939.....		\$ 7,209.16
Association assets, 1938.....	4,544.62	
Surplus, 1939.....	796.76	
	<hr/>	
Assets December 31, 1939.....		5,341.38
Convention Reserve Fund, 1938.....	1,000.00	
	<hr/>	
Assets December 31, 1939.....		1,000.00
<i>Cereal Laboratory Methods</i> Fund, 1938.....	1,284.16	
Surplus, 1939.....	176.47	
	<hr/>	
Assets December 31, 1939.....		1,460.63
Decennial Index Fund Assets, 1938.....	150.00	
Surplus, 1939.....	150.00	
	<hr/>	
Assets December 31, 1939.....		300.00
	<hr/>	
TOTAL ASSETS DECEMBER 31, 1939.....		\$15,311.17

**FINANCIAL STATEMENT DECEMBER 31, 1939****ASSETS**

Manufacturers Trust Company checking account . . . . .	\$ 4,304.39
Petty Cash Fund, Lincoln, Nebraska . . . . .	201.62
Emigrant Industrial Savings Bank, New York . . . . .	868.37
Franklin Savings Bank, New York . . . . .	532.57
Building & Loan Stock, Kansas City <sup>1</sup> . . . . .	1,000.00
U. S. Treasury Bonds . . . . .	2,000.00
U. S. Saving Bonds . . . . .	6,120.08
1939 income receivable . . . . .	284.14

GROSS AND NET ASSETS . . . . . **\$15,311.17**

NO LIABILITIES

**REPORT OF THE AUDITING COMMITTEE**

We have examined the books and the report of the Treasurer for the year 1939 and to the best of our knowledge and belief, these are a true and accurate account of the receipts and expenditures of the American Association of Cereal Chemists.

W. R. STOKES, *Chairman*  
CHAS. A. GLABAU  
R. T. BOHN

**CEREAL CHEMISTRY FINANCIAL STATEMENT—1939****JOURNAL INCOME**

Membership			
Active 576 @ . . . . .	\$ 3.50	\$2,016.00	
Sustaining 54 @ . . . . .	10.00	540.00	
			\$ 2,556.00
Subscriptions			
Foreign 354 . . . . .	\$2,176.69		
Domestic 164 . . . . .	971.90		
		3,148.59	
Advertising . . . . .	2,245.37		
Accts. receivable . . . . .	126.30		
		2,371.67	
Reprints . . . . .	732.94		
Accts. receivable . . . . .	133.17		
		866.11	
Back issues . . . . .	603.32		
Accts. receivable . . . . .	24.67		
		627.99	
			\$7,014.36
1938 accts. receivable collected 1939 . . . . .			326.79
			<b>7,341.15</b>

**OTHER INCOME**

Interest on invested funds . . . . .	110.35
Cincinnati Convention surplus . . . . .	339.63
Amt. from General Association Fund . . . . .	200.00
	<b>649.98</b>
<b>TOTAL INCOME . . . . .</b>	<b>\$10,547.13</b>

<sup>1</sup> Carried on books at value authorized in 1936; now represented by \$400 class A stock plus accumulated interest of \$30.89 plus \$1,600 of class B certificates of undetermined value.

## JOURNAL EXPENSE

Journal issues.....		\$ 5,536.08
Reprints.....		654.76
Salaries		
Editor-in-Chief.....	\$ 499.98	
Assistant Editor.....	499.98	
Managing Editor.....	343.16	
Stenographer.....	390.00	
		<u>1,733.12</u>
Petty Cash		
Petty cash 1938 balance.....	\$199.18	
P.C. reimbursements 1939.....	325.00	
		<u>\$ 524.18</u>
Less P.C. 1939 balance.....	201.62	
		<u>\$ 322.56</u>
Gross petty cash expenditures 1939.....		\$ 322.56
Less 1938 accts. payable paid in 1939.....		6.34
		<u>316.22</u>
Net petty cash expenditures.....		316.22

## OTHER EXPENSE

Decennial Index Fund.....		75.00
		<u>\$ 8,315.18</u>
TOTAL EXPENSE.....		\$ 8,315.18
GROSS PROFIT.....		\$ 2,231.95
Less 1938 accounts receivable collected 1939.....		326.79
Less 1938 accounts uncollectible.....		6.46
		<u>\$ 1,898.70</u>
NET PROFIT 1939.....		\$ 1,898.70

We have examined the books of the Managing Editor of *Cereal Chemistry* for the calendar year 1939 and find the same to be correct to the best of our knowledge.

H. H. JOHNSON  
C. W. OFELT

# CEREAL CHEMISTRY

VOL. XVII

MAY, 1940

No. 3

## A NEW FLOUR TEST: THE "DEXTRIN FIGURE"

D. W. KENT-JONES and A. J. AMOS

The Dover Laboratories, Dover, England

(Received for publication October 14, 1939)

One of the main objects of cereal chemistry in recent years has been the evolving of tests capable of revealing to the miller and to the baker defects in his flour which would ultimately show up as faults when that flour was made into bread, cakes, etc. One such defect for which there has been no satisfactory test in the past is the tendency of some flours to give rise to stickiness and undue softness or dampness in the crumb of the baked goods. The present investigation was, therefore, undertaken in the hope that a method might be devised which would permit the determination of the extent to which a flour is likely to give rise to stickiness and streaks in the crumb.

This particular fault is not restricted to yeast-fermented goods but is encountered in goods which are aerated by other means; as a matter of fact, it has been particularly prevalent in recent years in the so-called "soda bread" which constitutes something like 60% of the bread produced in Eire. This bread is produced by adding soured milk and sodium bicarbonate to the flour (which often contains some monocalcium phosphate) to make a slack dough and then baking this in special iron pots at a relatively low temperature for a comparatively long time. The baking is often accomplished on a peat fire and the lid is also covered with glowing peat, a procedure which corresponds approximately to an oven temperature of 390°–440°F. For large loaves the actual baking may proceed for as long as 1½ hours. The flour for this purpose is milled from a grist of soft weak wheats and the trouble of sticky crumbs in the resultant bread has naturally been much more prevalent since the Irish millers have been obliged by law to employ a considerable proportion of native wheat, much of which has been somewhat out-of-condition and sprouted.

The same trouble of stickiness and streaks in the cooked goods is also experienced in the case of self-raising flour, which has become increasingly popular in recent years. Since in England the native wheat is often a constituent of the grist for such flour, the trouble is

more common in those years when the harvest has been a wet one resulting in the presence of sprouted kernels in many samples of English wheat.

In the past, one test has provided a useful, although by no means infallible, guidance concerning the likelihood of flours to lead to stickiness in the crumb of the baked goods, although the test was originally devised and later modified for quite different purposes. The test in question is the maltose test which was devised by Rumsey (1922) as a measure of one factor of flour strength but was later modified by Kent-Jones (1924, p. 255) who then employed it as an index of the gas producing power of flour (p. 264). This latter worker, who determined the amount of maltose present after incubation by means of Fehling solution and the methylene blue titration method of Lane and Eynon (1923) and expressed the results on a percentage basis, found that the maltose figures of English commercial baking flours ranged from less than 1% to rather more than 3%. He suggested that, if a flour is to gas satisfactorily during fermentation, it should have a maltose figure not lower than 1.6% (later modified—1927, p. 360—to 1.5%) but he also suggested (1927, p. 361) an upper limit of 2.3%, since it had been found that flours possessing maltose figures higher than this were very liable to develop stickiness in the crumb of the loaf or other baked goods. The underlying principle of the maltose test, namely the measurement of the extent of diastatic action upon the starch of the flour concerned and not upon specially prepared starch which may have become modified in nature, is now well established and, although in Britain and indeed in Europe generally the procedure suggested by Kent-Jones is commonly used and the results expressed as a percentage, in America the method of Blish and Sandstedt (1933) is more commonly followed. In this latter method the flour is incubated with a buffer solution at a temperature of 30°C. and the maltose determined through its reducing action on ferricyanide in alkaline solution; the results are expressed as milligrammes of maltose formed from 10 g. of flour (Rumsey units).

Numerous investigations of the maltose test have been undertaken but these have been mainly concerned with the correlation between diastatic activity and gas production or with the comparison of results obtained by different methods. Attention may be drawn to the following: Malloch (1929); Blish, Sandstedt, and Astleford (1932); Davis and Worley (1934); Eva, Geddes, and Frissell (1937); Davis (1937); Bottomley (1938). The general opinion of workers has been that, while there is not an exact relationship between maltose figure and gas production, the maltose figure does nevertheless give useful guid-

ance concerning the general gassing power of a flour and its liability to give rise commercially to defects due to incorrect diastatic activity. Kent-Jones (1938, p. 580) stated that "Nevertheless, in general commercial experience, the value of the maltose test will be confirmed by most workers, and it is generally dangerous to have too low a maltose figure as then there may be lack of gassing power under some systems of fermentation and there is the possibility of pale crust." Fisher, Halton, and Hines (1938), however, differed from a number of previous workers in that they could not in their tests obtain even a reasonable correlation between maltose figure and gas production and they concluded that the real value of the test lay in the upper limit of the scale. They stated that flours with maltose figures above 2.3%—the upper limit suggested by Kent-Jones (1927)—gave sticky doughs, the loaves refusing to bake. This statement of these authors is, however, too definite since, although a high maltose figure is often accompanied by the production of stickiness in the crumb, it has been found that there are many exceptions and a high maltose figure, therefore, is not an absolute index that this trouble will arise and, in fact, cannot be regarded as other than a warning of the possibility of stickiness.

Sherwood and Bailey (1926) and Kent-Jones (1927, p. 361) suggested that this trouble of stickiness in the crumb might be connected with excessive proteoclastic activity but Kozmin (1933) refuted this theory and stated that the trouble was due to excessive enzymic splitting up of the starch during the baking process. The resultant stickiness she attributed to the fact that, on account of the excessive enzymic action, insufficient starch remained to hold the water. Kozmin stated that the enzymic decomposition of the starch resulted in the production of dextrans in addition to sugar and suggested that a new criterion of flour quality—dextrinising capacity—was needed. In the light of modern knowledge this work of Kozmin has particular merit since it is now known that what at one time was regarded as the diastatic enzyme comprises a group of enzymes and that of the two main members of the group—alpha-amylase and beta-amylase—the former converts starch into dextrin. These two enzymes have been studied by a number of workers in recent years and knowledge of their probable significance in panary fermentation has thereby become much clearer. Reference must be made to the work of Andrews and Baily (1934); Ougrimov (1935); Read and Haas (1936); Munz and Bailey (1937); Blish, Sandstedt, and Mecham (1937) and Blish, Sandstedt, and Kneen (1938). The various investigators reveal that beta-amylase converts starch into maltose and a dextrin,

the former predominating. This enzyme is, however, almost without action upon raw undamaged starch and in a flour only acts upon those granules which have been rendered susceptible to attack, usually through mechanical disintegration during the milling. Alpha-amylase exhibits a different mode of attack upon the starch molecule and produces, as a result of its action, dextrins. There is some uncertainty as to whether this enzyme alone is able to act upon raw undamaged starch (Blish, Sandstedt, and Kneen, 1938).

Alpha-amylase is more thermo-stable than beta-amylase and is particularly active at temperatures in the region of 64°C. Therefore, when the alpha-amylase activity of a flour is high, considerable dextrin formation will occur during the baking process; the rate of dextrin formation will continuously increase as the temperature of the dough rises to 64°C. and will be at a maximum at about this temperature. A given alpha-amylase activity will consequently have a greater detrimental effect, if the baking is performed at a relatively low temperature and is comparatively protracted. Under such conditions the temperature of the dough will remain in the region of 64°C. for a longer period and a greater time will elapse before the dough reaches the temperature at which enzymic activity is inhibited. This is a further reason why, if the trouble of sticky crumbs is due to alpha-amylase activity, it should be particularly prone to occur in soda bread which, as explained previously, is baked at a comparatively low temperature for a long time. Similar considerations naturally apply to other breads and goods subjected to similar baking conditions.

The above considerations reveal that there is a very definite need for a test which will provide a measure of the alpha-amylase activity of a flour. Obviously, in devising such a test it is essential to follow the principle adopted in the maltose test and to ensure that the enzyme is allowed to act upon the starch of the flour itself and not upon an artificially prepared or soluble starch. It is realised, of course, that closely allied enzymes, such as alpha- and beta-amylase, cannot be considered as functioning quite independently of one another, thus permitting the ready assessment of the activity of one of them entirely uninfluenced by that of the other. For the purposes of flour control work, however, it is only necessary to be able to measure reliably the quantity of dextrin formed under standardised and controlled conditions, provided the measurements so obtained can be shown to check up with the results actually obtained in baking. The method to be described is of this nature and after long experience in the authors' laboratory it has proved to be definitely useful in practice. It has revealed that, although the maltose figure has in the past proved a

useful general guide as to the probability of stickiness in the crumb, the maltose figure and alpha-amylase activity do not always run parallel and sometimes a distinctly high maltose figure may be accompanied by low alpha-amylase activity and *vice versa*.

### Details of the Test

Molin (1934) devised a method for the determination of sprout damage in wheat and rye in which the extent to which autolytic hydrolysis proceeded in a flour-water mixture at 62°C. was measured refractometrically. The temperature of 62°C. was selected as being in the region of the optimum for the dextrin-forming enzyme. Munz and Bailey (1937) introduced certain modifications into the technique. It has been the authors' experience, however, that when this method is applied to ordinary flours the variations in refractive index so obtained are not of sufficient magnitude to enable a satisfactory differentiation to be made between different flours. It seemed that the most logical way of attaining the object in view would be to perform a high-temperature incubation as suggested by Molin and then actually to determine the quantity of dextrin formed under these conditions. Edwards, Nanji, and Chanmugan (1938) have devised a procedure for the determination of dextrin in the presence of sugar and starch and it was, therefore, decided to attempt to combine the principle of this procedure with that of Molin's method.

It must be borne in mind that the aim of this investigation was to devise a comparatively simple analytical procedure which would enable reasonably valuable prognostications to be made concerning the behaviour of a flour when baked. In other words, provided the method permitted reasonable duplication and furnished results capable of significant interpretation, it would fulfil admirably the object in view and there would be no need to lengthen the investigation in order to introduce refinements which could serve no useful purpose. After numerous experiments and trials the following technique was finally adopted:

Weigh 1.25 g. of the flour under test into a dry clean boiling tube (6x1 inches) provided with a fairly thin glass stirring rod. Molin employed a bare stirring rod but the present authors have found that if a rubber-tipped one is used the stirring of the flour-water mixture into a perfectly smooth paste, which is of course an essential step in the process, can be accomplished with greater certainty and ease. Add to the flour in the tube 3 ml. of distilled water at room temperature and carefully stir the mixture into a thoroughly smooth paste. The authors have used distilled water as Molin did since it did not seem from the



work of Munz and Bailey (1937) that the employment of a buffer solution was necessary or even likely to be helpful. Particularly is this so in the present case where the ratio of water to flour is small and where, in any case, refinements possibly leading to a high degree of precision can afford no greater guidance commercially.

Place the tube containing the flour-water paste in a water bath maintained at a temperature of  $62^{\circ}\text{C} \pm 0.1^{\circ}\text{C}$ . The constancy of the temperature of incubation is, of course, extremely important and details of the construction of the bath and of the thermostatic arrangement are therefore given later. The time of incubation suggested by Molin was 10 minutes, but it was found that, employing ordinary boiling tubes which naturally vary slightly in wall thickness, etc., the adoption of such a period of heating resulted in a too marked variation between replicates. The duration of the time of incubation was accordingly lengthened to 30 minutes and this modification had the further advantage that it led to the production of a greater quantity of dextrin.

After an incubation of exactly 30 minutes, therefore, remove the tube from the heating bath and plunge it into a bath of cold water. Allow it to remain in this cold bath for four minutes. On no account must the flour-water paste be stirred or in any way disturbed between the time it is immersed in the hot-water bath and the completion of its four minutes of cooling. To the cooled paste add 2 ml. of distilled water and stir the mixture again into a smooth paste. Then add 20 ml. of water, stir the mixture thoroughly, and then centrifuge it.

To 10 ml. of the supernatant liquid add 2 ml. of *N*/10 iodine solution and enough alcohol-potassium acetate solution to give a total volume of 100 ml. (This latter solution consists of a mixture of equal volumes of alcohol<sup>1</sup> and distilled water to each 100 cc. of which mixture has been added 4 ml. of 10% potassium acetate solution.) Allow the solution to stand for 5 minutes or so and then filter off the precipitated starch iodide through a medium-sized Buchner funnel (about 4 in. diameter). For this filtration it is found most convenient to use a No. 5 Whatman filter paper covered with a thin layer of prepared asbestos fibre as used with Gooch crucibles.

Transfer 50 ml. of the clear filtrate to a glass evaporating dish and evaporate it down on a water bath to a volume of 5–6 ml. This evaporation must not be allowed to proceed too far as, if the volume becomes less than 5 ml., some material is thrown down as a rubbery encrustation which is not soluble on the addition of the small quantity of the alcohol presently to be added. A slight cloudiness at this stage

<sup>1</sup> In England when the test is used in routine work the alcohol is replaced by industrial spirit B.P. which is available to laboratories.

is, however, not important as this disappears when the first small quantity of alcohol is added.

Wash out the small volume of liquid from the evaporating dish into a 250-ml. beaker with about 10–15 ml. of 95% alcohol<sup>1</sup> and then add a further quantity of the alcohol, making a total volume of 100 ml.; some of this 95% alcohol<sup>1</sup> may be used for further washings of the evaporating dish, if necessary. The precipitate of dextrin is at first very fine but it gradually becomes more granular and settles. Allow the mixture to stand for at least one hour but, better still, overnight. Filter off the precipitated dextrin on a tared alundum crucible of medium porosity and wash with alcohol and finally with ether. Dry the precipitate at 100°C. for one hour and then weigh it. The result is reported to the nearest 0.5%.

It is fortunate that the presence of normal self-raising ingredients does not interfere with the determination and the technique can be applied without modification to self-raising and soda bread flours.

With respect to replication, it is usual for duplicate tests to vary from the mean by not more than  $\pm 0.5$  dextrin unit. In other words, a flour giving a dextrin figure of 7.5 should upon being retested afford a value lying between 7.0 and 8.0 dextrin units. Such a degree of accuracy is, of course, quite satisfactory for the routine testing of commercial flours.

It is, of course, of utmost importance that both the time and temperature of incubation given in the method are rigidly adhered to and it is accordingly necessary that the bath be so designed and constructed that the limits of variation of temperature from the chosen figure of 62°C. are  $\pm 0.1^\circ\text{C.}$  as suggested by Molin (1934).

In case it may be of help to workers who have no suitable bath available, details of the bath the authors have constructed are given. A wooden shell 16x20 inches by 13 inches deep was constructed and fitted with supporting pieces at the bottom and sides so as to receive a copper bath two inches smaller in each dimension. The space between the inner bath and the outer wooden shell was packed with granulated cork, and the top of this intervening space was sealed with copper sheeting affixed to both the bath and the wooden shell; this was done in order to prevent moisture from gaining access to the cork.

Across the middle of the top of the bath from back to front is fitted a copper-faced wooden bar which supports a toluol regulator, the necessary immersion heaters, the shaft of the stirrer and a thermometer. On either side of this bar is fitted a removable lid which comprises a two-inch-thick wooden box packed with granulated cork and faced on its underside with sheet copper. Where the heaters pass through the middle bar to dip into the water, a tight joint must

be made with bitumen or some such compound to ensure that the water vapour arising from the bath does not come into contact with the wires connecting the mains to the heaters. Unless this precaution is efficiently carried out, it is most probable that the bath and the water will become electrified and this may lead to serious accidents.

The stirrer is operated by an electric motor and the immersion heaters are supplied with current through a vacuum relay which is operated by the toluol regulator.

The authors' bath, constructed as above, has been in continuous use for more than six months and the temperature has not varied from the 62°C. by more than the suggested limits.

It will be found convenient to construct metal holders (cages) to take say six boiling tubes of the size mentioned. This enables a series of tests to be immersed in the incubating bath simultaneously and also to be removed from this bath and placed in the cooling bath all together without any loss of time. These cages are so constructed that the test-tubes can be secured in their respective positions and, when in place, are immersed to at least half their length.

### Examination of Results and Discussion

Having finally decided upon the technique of the proposed test, which it must be remembered is empirical in nature and, therefore, requires that the conditions laid down shall be rigidly adhered to, the authors applied the method to a large number of commercial flours which covered a wide range as regards liability to produce sticky crumbs. It is proposed to discuss a number of these results in this section of the paper. Before the method was employed with commercial flours, a series of preliminary experiments was performed in order to obtain an idea of the relationship between the magnitude of the dextrin figure and the degree of stickiness in the crumb, and the results of one of these experiments will be found in Table I. An

TABLE I

EFFECTS OF ADDITIONS TO FLOUR OF DIASTATIC ENZYME PREPARATION CONTAINING BETA- AND ALPHA-AMYLASE BUT PARTICULARLY HIGH IN ALPHA-AMYLASE ACTIVITY

Sample	Maltose figure	Dextrin figure	Nature of crumb of loaf
	%		
Control flour	1.83	5.5	Satisfactory
Control flour + 0.008% enzyme preparation	3.61	8.1	Satisfactory
Control flour + 0.016% enzyme preparation	4.80	13.0	Just sticky
Control flour + 0.032% enzyme preparation	5.32	19.0	Very sticky

enzymic preparation which, although containing some beta-amylase, was particularly active in alpha-amylase was obtained and was added in comparatively small proportions to an otherwise satisfactory flour. By this means it was possible to produce damp and sticky crumbs, thus confirming the general theme of this paper, and to obtain an idea of the correlation between the dextrin figure and crumb stickiness.

In Table II will be found a selection of the very large number of results which the authors have obtained by the application of this test

TABLE II  
DEXTRIN FIGURES, BAKING RESULTS, AND MALTOSE FIGURES OF A SERIES OF  
COMMERCIAL FLOURS

Lab. ref. no.	Maltose figure %	Dextrin figure	Nature of crumb of loaf
YEAST BAKING FLOURS			
PZ. 606	1.83	5.5	Satisfactory
YZ. 669	2.10	6.5	"
CZ. 599	2.53	6.0	"
K. 885	2.43	5.5	"
QZ. 810	3.34	17.0	Very sticky and damp
SODA BREAD FLOURS			
BZZ. 1220	1.18	5.0	Satisfactory
BZZ. 1290	1.00	6.0	"
SZZ. 1100	1.20	6.0	"
SZZ. 1101	1.13	6.5	"
SZZ. 1102	1.13	5.0	"
SZZ. 1108	1.09	7.5	"
XZZ. 1373	1.34	7.0	"
SZZ. 1093	1.13	8.0	"
SZZ. 1094	1.13	7.0	"
SZZ. 1095	1.00	9.0	"
OX. 154	1.61	7.5	"
CZZ. 670	1.40	8.5	"
YZ. 714	1.39	10.0	"
RZZ. 1188	1.34	10.0	"
XZZ. 1374	1.32	10.5	Border line case
XZZ. 1387	1.32	11.0	" " "
XZZ. 1383	1.60	12.0	" " "
XZZ. 1419	1.21	13.5	" " "
XZZ. 1415	1.25	11.5	" " "
XZZ. 1381	1.56	14.0	Damp and sticky
SZZ. 1125	1.69	12.5	" " "
FZ. 527	1.65	12.5	" " "
FZ. 574	1.77	14.0	" " "
EZ. 984	1.51	12.5	" " "
SZZ. 1113	1.33	15.0	Very damp and sticky
SZZ. 1116	1.36	15.5	" " " "
FZ. 575	2.15	18.0	" " " "
FZ. 576	2.32	19.5	" " " "
XZZ. 1209	1.34	17.0	" " " "

to commercial flours. The results have been arranged in four groups on the basis of the degree of stickiness found in the baked goods.

Before discussing the main implications of the table, attention is drawn to certain interesting results which reveal clearly that the maltose figure is not a reliable indication as to whether stickiness will or will not be present in the crumb of the baked goods. For instance, flours XZZ.1373 and XZZ.1209 both possess the same maltose figure of 1.34% but, whereas the former has a dextrin figure of 7.0 and bakes satisfactorily, the latter has a dextrin figure of 17.0 and produces a very sticky crumb. Then again, samples OX.154 and FZ.527 have essentially the same maltose figure (1.61% and 1.65% respectively) but they differ appreciably in alpha-amylase activity at 7.5 and 12.5 dextrin units respectively. Examining the matter from the other angle, it will be seen that flours CZ.599 and BZZ.1290 reveal identical alpha-amylase activities (dextrin figures of 6.0) but, whereas the latter flour has a very low maltose figure of 1.0%, the other sample has a very high maltose figure of 2.53%.

Thus, although the table reveals that a low maltose figure generally means that the crumb of the goods will be satisfactory and free from stickiness, yet, owing to the fact that the alpha-amylase and beta-amylase activities obviously do not always run parallel, a flour may have a low maltose figure and yet give a high dextrin figure and, in such cases, it is likely that the baked goods will prove unsatisfactory. Samples XZZ.1209, SZZ.1116, and SZZ.1113 in Table II are a few illustrative examples of this type of flour; they have low maltose figures at 1.34%, 1.36%, and 1.33% respectively, whereas their dextrin figures are high at 17.0, 15.5, and 15.0 respectively. Alternatively, a high maltose figure does not necessarily entail a high alpha-amylase activity as is seen in the case of samples YZ.669, CZ.599, and K.885 recorded in Table II. These flours all possess high maltose figures at 2.10%, 2.53%, and 2.43% respectively but nevertheless are low in alpha-amylase activity as revealed by the low dextrin figures of 6.5, 6.0, and 5.5 respectively. Thus, although these samples possessed high maltose figures, they did not and would not give rise to sticky and damp crumbs.

It will be realised that with any kind of bread fault arising from the nature of the flour there must be borderline cases. In other words, it is impossible with any type of defect to devise a test which will provide a precise limit above or below which the flour will be satisfactory or unsatisfactory as the case may be. Flours possessing values in the region of the suggested limit may give either satisfactory or unsatisfactory results according to the conditions to which they are

subjected in the baking test. If, however, a flour possesses a value in the region of the limit, it must be definitely suspect and considered as liable to give complaints, although not necessarily bound to do so.

The general experience of this test based upon the great number of baking tests made to check up the correlation between the dextrin figure and the occurrence of stickiness in the crumb similar to those reported in Table II, suggest that, if a flour intended for the production of Irish soda bread or self-raising goods is to be perfectly satisfactory and not in any way liable to give stickiness in the crumb, then the dextrin figure should be below 10.0. The further the figure is below 10.0, the more satisfactory the flour is from this point of view. Many really good flours give dextrin figures around 5.0–6.5 or even lower.

Dextrin figures over 10.0 indicate that the flour is capable of giving some stickiness and clamminess in the crumb if the baking conditions are adverse, as, for example, when the baking temperature is low and the time of baking prolonged. It may happen that no commercial complaints are received about a flour possessing a figure somewhat above 10.0 but such a figure is nevertheless a danger signal and the flour certainly cannot be considered as completely safe and free from capability of causing the type of trouble being discussed.

Experience has shown that the more the dextrin figure is above 10.0 the greater the danger of trouble, and really high figures such as 16.0–20.0 or even higher, which are sometimes encountered (see flours at end of Table II) indicate that the flour is likely to give trouble of stickiness in the crumb under almost all baking conditions, whether favourable or not.

It is difficult to forecast the behaviour of a flour which has a dextrin figure between 10.0 and 14.0. It will depend entirely upon the conditions. It is quite possible that a flour with a dextrin figure of 12.5 may prove satisfactory while one with a figure of 11.5 may lead to a complaint. The reason for such a happening is that the flour with a figure of 12.5 has been baked under favourable conditions, while the flour with a figure of 11.5 has been baked under adverse conditions, *i.e.* those which have afforded greater scope for alpha-amylase activity. Thus the data of Table II show that flour XZZ.1419 with a dextrin figure of 13.5 did not happen to give such a poor baking result as regards stickiness of crumb as flour EZ.984 with the slightly lower dextrin figure of 12.5. In a controlled experiment, of course, in which both flours had exactly the same baking, that with the higher dextrin figure would tend to give the worse loaf. This would be seen more

especially when the actual conditions chosen were difficult ones such as long baking at a low temperature.

The suggestions given above concerning the limits for dextrin figures refer to flours intended for the production of soda bread or of self-raised goods and do not necessarily apply to yeast baking flours. Limits for such flours have not been suggested since, at the moment, we have not had sufficient commercial yeast baking flours high in alpha-amylase activity to enable correlation tests to be made. It may be that a given dextrin figure would be less likely to cause trouble in yeast bread than in soda bread, owing to the fact that the former process involves baking at an appreciably higher temperature for a distinctly shorter period and thus considerably curtails the activity of the alpha-amylase. This dextrin test would have been useful in 1926 when the Manitoba crop caused considerable difficulties in England from stickiness of crumb (Kent-Jones 1927, p. 361). Under such conditions the limits for the dextrin figures of yeast baking flours could soon be established.

Often goods of the pudding or dumpling type are made from self-raising flour, and when the flour is unsatisfactory these dumplings are heavy and dough-like when cut instead of being light and mealy. Indeed, the boiling of dumplings is often used in England for testing self-raising flours. This method can, of course, with advantage be replaced by the dextrin test which gives a quantitative result and permits a better differentiation between individual flours.

The dextrin figure test can also be applied to wheat. In this case, the wheat should be milled on a laboratory mill under standard conditions and the test applied to the resultant flour. It is advisable to make one or two tests upon laboratory milled and commercially milled flour from the same wheat in order to ascertain the relationship between the two. When sprouted or out-of-condition wheat has to be used, the application of this test will be found of value in deciding the proportion of such wheat which can be employed with reasonable safety.

There seemed another possibility for the utilisation of this test as a means of reducing the liability for trouble to occur when, owing to circumstances, a proportion of sprouted or out-of-condition wheat has to be employed. The dextrin figure of each of the flour streams in a mill grinding a grist containing sprouted wheat was determined and the tests revealed that certain streams were particularly high in alpha-amylase activity. In some cases, it might be possible to segregate such stocks. Certain preliminary work has been accomplished in this direction but the mills investigated have been too few to

warrant any very definite conclusions. It may be stated, however, that so far the first break flour has possessed a higher alpha-amylase activity than any other flour stream.

Certain mechanical tests have been suggested as a means of assessing the alpha-amylase activity of flour. These depend upon a mechanical measurement of the viscosity of a gelatinised starch paste resulting from the gradual heating of a flour suspension under carefully controlled conditions which permit appreciable enzymic conversion of starch. It would appear, however, that the reading obtained (conveniently obtained in the form of a graph) is dependent upon the amount of starch which has escaped enzymic conversion and thus remains for gelatinisation and is not an index of the path by which the starch has been converted.

In other words, such an instrument essentially measures the amount and not the nature of the starch conversion and if, for example, a given proportion of the starch is turned into maltose the results, as far as the viscosity curve is concerned, will be essentially the same as if the same proportion of the starch were converted into dextrin; the nature of the baked goods in the two cases might, however, be very different. The authors have investigated this method and find that it usually gives a useful general guide as to the behaviour of the flour just as does the maltose test but, in certain cases, such as with flours possessing high maltose figures but low dextrin figures, the results are liable to be misleading. It is suggested, therefore, that the method described in this paper in which the actual dextrin produced by the action of the alpha-amylase is determined is much sounder in principle and more informative and accurate.

### Summary

A method of assessing the alpha-amylase activity of a flour by the determination of a "dextrin figure" is described which, it is suggested, furthers knowledge of the relationship of the enzymic activity of flour and wheat to practical baking problems and which should prove of practical value to the milling and baking industries.

This dextrin figure reveals the extent to which a flour is likely to give rise in the baked goods to those defects normally associated with the use of appreciable proportions of sprouted wheat, namely dampness and stickiness in the crumb.

The majority of flours milled from sound wheats give dextrin figures well below 10.0.

Flour possessing dextrin figures in the region of 10 to 14 may be considered as suspect. They may or may not give trouble, depending



upon the baking conditions; prolonged baking in a slack oven will be likely to result in stickiness, while rapid baking in a hot oven will tend to produce satisfactory results.

Flours possessing dextrin figures much over 14 are likely to give trouble commercially, whatever the baking conditions.

The test has been used as a routine one by the authors for approaching a year and, from the hundreds of results now obtained, they are satisfied that it is of great value for determining the liability of a flour to give rise to that fault which is attributed to excessive alpha-amylase activity, namely sticky and damp crumbs.

#### Acknowledgments

The authors wish to acknowledge the assistance they have received in compiling the analytical data in this paper from the following members of the staff of these laboratories: E. R. S. Winter, W. Martin, and R. C. Spalding.

#### Literature Cited

- Andrews, J. S., and Bailey, C. H.  
1934 The amylases of normal and germinated wheat flours. *Cereal Chem.* **11**: 551-556.
- Blish, M. J., and Sandstedt, R. M.  
1933 An improved method for the estimation of flour diastatic value. *Cereal Chem.* **10**: 189-202.
- Blish, M. J., Sandstedt, R. M., and Astleford, G. R.  
1932 Sugars, diastatic activity and gassing power in flour. *Cereal Chem.* **9**: 378-393.
- Blish, M. J., Sandstedt, R. M., and Kneen, E.  
1938 The cereal amylases with reference to flour and malt behavior. *Cereal Chem.* **15**: 629-657.
- Blish, M. J., Sandstedt, R. M., and Mecham, D. K.  
1937 Action of wheat amylases on raw wheat starch. *Cereal Chem.* **14**: 605-628.
- Bottomley, R. A.  
1938 The relationship between diastatic activity (maltose figure) and "gassing power" of experimentally milled flours from some Australian wheats. *Cereal Chem.* **11**: 509-520.
- Davis, C. F.  
1937 A collaborative study of the Blish-Sandstedt, Schoorl, and Bertrand methods for determining reducing sugars in flour diastatic activity measurements. *Cereal Chem.* **14**: 74-85.
- Davis, C. F., and Worley, D. F.  
1934 The correlation between diastatic activity and gassing power in commercial flours. *Cereal Chem.* **11**: 536-545.
- Edwards, F. W., Nanji, H. R., and Chanmugan, W. R.  
1938 A new iodine method for the determination of starch. IV. The determination of dextrins in the presence of starch and sugars. *Analyst* **63**: 697-702.
- Eva, W. J., Geddes, W. F., and Frisell, B.  
1937 A comparative study of various methods of measuring flour gassing power. *Cereal Chem.* **14**: 458-480.
- Fisher, E. A., Halton, P., and Hines, S. F.  
1938 Some observations on maltose production in flour and dough. *Cereal Chem.* **15**: 363-377.
- Kent-Jones, D. W.  
1924 Modern cereal chemistry (1st ed.).  
1927 Modern cereal chemistry (2nd ed.).  
1938 Modern cereal chemistry (3rd ed.).

- Kozmin, N. P.  
1933 Biochemical characteristics of dough and bread from sprouted grain. *Cereal Chem.* **10**: 420-436.
- Lane, J. H., and Eynon, L.  
1923 Determination of reducing sugars. *J. Soc. Chem. Ind.* **42**: 32T.
- Malloch, J. G.  
1929 Modification of Rumsey's method for the determination of diastatic activity in flour. *Cereal Chem.* **6**: 175-181.
- Molin, G.  
1934 Determination of sprout damage in wheat and rye by means of the dipping refractometer. *Cereal Chem.* **11**: 153-159.
- Munz, E., and Bailey, C. H.  
1937 Effect of the enzymes of a malted wheat upon certain properties of flour dough. *Cereal Chem.* **14**: 445-457.
- Ougrimov, P. S.  
1935 *Biochem. Z.* **282**: 74.
- Read, J. W., and Haas, L. W.  
1936 Further studies on the baking quality of flour as affected by certain enzyme actions. Relative starch liquefying power of the diastatic agents investigated. *Cereal Chem.* **13**: 14-37.
- Rumsey, L. A.  
1922 Diastatic enzymes of wheat flour and their relation to flour strength. *Am. Inst. Baking, Bul.* **8**.
- Sherwood, R. C., and Bailey, C. H.  
1926 Control of diastatic activity in wheat flour. II. Experiments with flour milled on a commercial scale. *Cereal Chem.* **3**: 163-182.

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## FRESH, FROZEN AND DRIED EGGS AND EGG PRODUCTS (THEIR USES IN BAKING AND FOR OTHER PURPOSES)<sup>1</sup>

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(Received for publication August 21, 1939)

Hen eggs are available to manufacturers of food products in four forms, namely shell eggs, liquid eggs, frozen eggs, and dried eggs. The separated whites and yolks are also available for commercial uses in liquid form, or frozen, or dried. The baker, who, among food manufacturers, is the largest consumer of fresh and frozen eggs, utilized in 1932 over 11 million dollars worth of fresh shell eggs, nearly 21 million dollars worth of frozen egg whites, and 15 million dollars worth of frozen yolks (Bailey and LeClerc, 1935). In some of his products, *e.g.*, sponge cake, the cost of eggs or egg products makes up fully 70% of the total cost of ingredients used. Eggs constitute the greater portion of the cost of ingredients in cake making, in general; it is estimated that approximately one-half the cost of all the ingredients used by the cake baker is that of eggs or egg products—a total of nearly \$50,000,000 being spent by the baker for these products.

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<sup>1</sup> Food Research Division Contribution No. 451.

The manufacturer of noodles, the value of whose product amounts to over \$10,000,000, is also one of the large users of eggs, especially egg yolk (frozen or dried) and whole eggs (fresh, frozen or dried). About 5½% of the weight of noodles must be made up of whole eggs and/or of yolk and the value of egg products used in making noodles is fully \$1,500,000 a year.

In recent years, the prepared-flour industry has been making notable strides. Dried eggs are largely used as one of the essential ingredients of most prepared mixes.

The quantity of yolk (largely frozen yolk) used by the mayonnaise<sup>2</sup> and salad-dressing industries is quite considerable, approximating in 1937 some 15,000,000 to 20,000,000 pounds (Radabaugh, 1938).

### **Importance—Historical—Statistics**

In 1924, the first year for which reliable statistics regarding egg production in this country are available, over 1,900 million dozens were produced, valued at nearly \$550,000,000. The hen population of the United States is now approximately 500 million; the average number of eggs laid per hen is five dozen per year. Eggs are produced by fully 90% of our farmers and the income from this source alone is about 3½% of the total cash receipts of the farmers.

The chief egg-producing centers are situated within the so-called "North Central States," which supply 85% of the eggs that are subsequently frozen and used chiefly by bakers.

### **Uses for Eggs in the United States**

Estimates for 1935, based upon information furnished by the Poultry Section of the Agricultural Adjustment Administration, are shown in Tables I and II. (Total eggs used are estimated at 2,764 million dozens.)

### **Frozen Eggs**

About 30 years ago H. J. Keith began freezing eggs commercially in St. Paul, Minnesota, although the freezing of eggs is said to have been started in Kansas some time previously. China, however, was the first country not only to freeze eggs on a large scale but also to dry them. By 1921, China was exporting to the United States alone some 18 million pounds of frozen eggs, produced chiefly in such centers as Shanghai, Nanking, Hankow, Tientsin, and Tsintao. The production of frozen eggs in the United States at that time was about 46 million

<sup>2</sup> "The semisolid emulsion of edible vegetable oil, egg yolk, or whole egg, a vinegar, and/or lemon juice, with one or more of the following: salt, other seasoning commonly used in its preparation, sugar and/or dextrose. The finished product contains not less than 50 percent of edible vegetable oil." (U. S. Department of Agriculture Service and Regulatory Announcements, 1936.)

TABLE I  
USES FOR EGGS

	<i>Million dozens</i>	<i>Percent</i>
<b>Egg Distribution</b>		
Shell egg consumption (fresh and storage)	2,285	82.6
Liquid or frozen egg	187	6.8
Dried egg	42	1.5
Used for hatching	113	4.1
Loss as rots, broken, etc.	137	5.0
<b>Shell Egg Uses</b>		
Table use	2,132	93.3
Bakery products	20	0.9
Hatching	113	4.9
Other uses	20	0.9
<b>Liquid and Frozen Eggs</b>		
Whole or mixed	93	49.7
Whites	48	26.3
Plain yolks	22	11.6
Salt yolks	10	5.2
Sugar yolks and/or glycerin yolks	14	7.2
<b>Whole or Mixed Eggs</b>		
Bakery products	86	92
Salad dressing	3	3
Noodles	2	2
Ice cream, etc.	2	2
<b>Whites</b>		
Bakery products	37	77
Candies, etc.	10	21
Ice cream	1	2
<b>Plain Yolks</b>		
Table use	0.4	2
Bakery products	7.1	32
Mayonnaise	6.6	30
Salad dressing	2.2	10
Noodles	4.8	22
Ice cream, etc.	0.7	3
Other uses	0.2	1
<b>Salt Yolks</b>		
Mayonnaise	7.5	75
Salad dressing	2.5	25
<b>Sugar and Glycerin Yolks</b>		
Table use	0.7	5
Bakery products	10.0	71
Mayonnaise	1.5	11
Salad dressing	0.4	3
Ice cream, etc.	1.4	10

TABLE I—*Continued*

	<i>Thousand dozens</i>	<i>Per cent</i>
<b>Dried Eggs</b>		
Dried whole egg	840	2
Dried whites	2,100	5
Dried yolks	39,060	93
<b>Dried Whole Egg</b>		
Bakery products	630.0	75
Mayonnaise	8.4	1
Noodles	33.6	4
Ice cream, etc.	8.4	1
Food beverage powders	25.2	3
Prepared puddings	16.8	2
Prepared flours	84.0	10
Dog food	12.6	1.5
Bird food	12.6	1.5
Fox food, egg shampoo, etc.	8.4	1
<b>Dried Whites</b>		
Bakery products	325.5	15.5
Candies, etc.	1,050.0	50.0
Prepared meringues, etc.	346.5	16.5
Prepared flours	42.0	2.0
Leather and fur trade	42.0	2.0
Lithographing	94.5	4.5
Cementing cork to bottle caps	52.5	2.5
Pharmaceuticals	21.0	1.0
Textile printing	105.0	5.0
Photography, ink, paints, etc.	21.0	1.0
<b>Dried Yolks</b>		
Bakery products	18,358.2	47
Mayonnaise	1,562.4	4
Noodles	2,343.6	6
Ice cream, etc.	11,718.0	30
Food beverage powders	390.6	1
Prepared puddings	390.6	1
Prepared flours	3,906.0	10
Other uses	390.6	1
<b>Eggs for Hatching</b>		
Commercial hatching	62,489.0	55.3
Farm hatching	50,511.0	44.7
<b>Loss—Broken, Rots, etc.</b>		
Leather and fur trade	6,850.0	5
Loss or waste	130,150.0	95

pounds. The freezing of eggs on an industrial scale in this country was at first confined largely to such market centers as Chicago, New York, Philadelphia, and Boston.

According to Heitz (1929), the first report on the quantity of frozen eggs produced in this country was in 1916, the quantity being 6½

TABLE II  
USES FOR EGGS

Uses	Per- cent of total	Shell eggs	Liquid and frozen					Dried			Total
			Whole or mixed	Whites	Plain yolks	Salt yolks	Sugar or glycerin yolks	Whole	Whites	Yolks	
		Thous. doz.	Thous. doz.	Thous. doz.	Thous. doz.	Thous. doz.	Thous. doz.	Thous. doz.	Thous. doz.	Thous. doz.	Thous. doz.
Table use	80.46	2,132,000	—	—	400	—	700	—	—	—	2,133,100
Noodles	.35	—	2,000	—	4,800	—	—	34	—	2,344	9,178
Bakery products	6.77	20,000	86,000	37,000	7,100	—	10,000	630	326	18,358	179,414
Salad dressings	.31	—	3,000	—	2,200	2,500	400	—	—	—	8,100
Mayonnaise	.65	—	—	—	6,600	7,500	1,500	8	—	1,562	17,170
Ice cream, etc.	.63	—	2,000	1,000	700	—	1,400	8	—	11,718	16,826
Confectionery	.42	—	—	10,000	—	—	—	—	1,050	—	11,050
Prepared flour	.15	—	—	—	—	—	—	84	42	3,906	4,032
Food beverage products	.02	—	—	—	—	—	—	25	—	391	416
Prepared puddings and meringues	.03	—	—	—	—	—	—	17	347	391	755
Bird and animal food, etc.	x	—	—	—	—	—	—	34	—	—	34
Pharmaceutical and photographical	x	—	—	—	—	—	—	—	42	—	42
Industrial	.27	6,850	—	—	—	—	—	—	295	—	7,145
Hatching	4.26	113,000	—	—	—	—	—	—	—	—	113,000
Other uses	.77	20,000	—	—	200	—	—	—	—	391	20,591
Losses—rots, broken, etc.	4.91	130,150	—	—	—	—	—	—	—	—	130,150
Total	100.00	2,422,000	93,000	48,000	22,000	10,000	14,000	840	2,102	39,061	2,651,003
Percent of total		91.36	3.51	1.81	0.83	0.38	0.52	0.03	0.08	1.48	100.00

x = less than 0.01%.

million pounds. By 1921 the production of frozen eggs had expanded beyond the confines of the big market centers, and by 1926 fully three times as many eggs were frozen in the country at large as in the big market centers mentioned above.

By 1926 over 50 million pounds were frozen and in 1927 81 million pounds, an amount equivalent in terms of fresh eggs to 2,500,000 cases or to approximately one-fourth the quantity of shell eggs stored. In 1927 over half the frozen eggs were stored in the Middle Atlantic, Eastern, and Northern Central states. In 1930, 180 million dozen eggs were "broken" in the United States and either frozen or sold as "liquid eggs."

By 1928 the production of frozen eggs in this country had increased to 148 million pounds, whereas the imports from China had fallen to about 8 million pounds. In 1937 some 225,000,000 pounds of frozen eggs were produced in the United States, of which 80,100,000 pounds were whole eggs, 18,600,000 pounds mixed eggs, 66,100,000 pounds whites, 16,000,000 pounds plain yolks, 26,700,000 pounds sugar yolks, 16,200,000 pounds salt yolks, and 336,000 pounds glycerin yolks. "Mixed" eggs are the liquid whole eggs which have been churned to break the vitellin membrane surrounding the yolks, run through sieves to incorporate intimately the yolks and whites, and then solidly frozen in cans. They are essentially "whole" eggs.

For many years no particular care was taken to select only the best eggs for freezing purpose, the chief object being to utilize the dirties, checks, cracks, leakers, etc., and thus to avoid monetary losses.

The eggs used for freezing at present are the best found on the market, being carefully selected to include only absolutely fresh eggs and thoroughly tested by candling before being broken. Today about 100 firms freeze eggs as their main, if not only, product (Anon., 1938), the eggs being for the most part (80% at least) from "current receipts" and laid during the spring months (middle of March to middle of June). Formerly about two-thirds of the breaking stock consisted of ungraded eggs purchased in local markets and one-third of "under-grades" purchased in central markets (U. S. Tariff Com., 1931). Frozen eggs are for most purposes as good as and often better than many of the so-called "fresh shell eggs." Owing to the extreme precautions taken to utilize, for freezing purposes, only sound fresh eggs, the frozen product contains as a rule far fewer bacteria than are found in the average "fresh" shell eggs (Bordas, 1922):

In general, frozen eggs do not compete with other frozen foods as they are a specialized type of product used chiefly by bakers and by mayonnaise, ice-cream, and noodle manufacturers.

Incidentally, it may be of interest to know that the annual per-capita consumption of eggs in this country is about 200. On the other hand, our northern neighbor, Canada, is the world's largest consumer with a per-capita consumption of over 330 (McMillen, 1930).

TABLE III<sup>1</sup>

STATISTICS ON DOMESTIC EGGS PRODUCED AND BROKEN FOR FREEZING  
AND IMPORTS OF EGG PRODUCTS

Year	U. S. prod. of shell eggs	Domestic eggs broken for freezing	Imports—equivalent in shell eggs			
			Frozen	Dried		Frozen and dried
				Egg albumen	Yolks and whole eggs	
	<i>Million dozens</i>	<i>Million dozens</i>	<i>1000 dozens</i>	<i>1000 dozens</i>	<i>1000 dozens</i>	<i>1000 dozens</i>
1921	—	39.4	15,684	16,538	18,196	50,418
1922	—	42.2	14,586	20,580	18,659	53,825
1923	—	60.9	5,736	17,057	7,835	30,628
1924	1,913	49.2	9,966	18,440	12,528	40,934
1925	2,003	67.4	19,224	19,710	18,380	57,314
1926	2,120	78.9	15,872	21,637	15,244	52,753
1927	2,162	110.7	6,150	21,074	8,819	36,043
1928	2,171	126.9	6,998	17,220	10,954	35,172
1929	2,145	133.0	13,310	21,866	14,962	53,138
1930	2,163	158.6	6,836	21,467	15,792	44,095
1931	2,870	130.0	672	18,272	20,342	39,286
1932	2,692	118.0	362	9,313	1,699	11,374
1933	2,652	147.0	354	6,381	3,681	10,416
1934	2,584	170.0	390	2,938	5,178	8,506
1935	2,618	177.0	1,026	13,698	11,011	25,735
1936	2,650 <sup>2</sup>	178.0	690	17,214	12,876	30,780
1937	2,625 <sup>2</sup>	246.0	1,293	20,762	14,293	36,348

<sup>1</sup> U. S. Tariff Com. Rep. No. 25, Dried Egg Products, Second Ser. (1931); and B. A. E. (unpublished data).

<sup>2</sup> Preliminary

### Composition of Whole Eggs, Whites, and Yolks

According to Langworthy (1901), the average shell eggs as purchased consist of 10.5% shell, 57.9% white, and 31.6% yolk. On the basis of the edible portion, eggs are composed of approximately 65% white and 35% yolk. These figures may vary considerably, for laboratory experiments with rather small, strictly fresh eggs show the following:

6 yolks = 103 cc. = 93 g. = 38.9%

6 whites = 166 cc. = 146 g. = 61.1%

V. S. Asmundson (1931) of the Department of Genetics, University of Wisconsin, weighed 707 eggs from 67 hens, the results being as follows:



Average weight of egg	53.6 g.
Average weight of yolk	16.1 g.
Average weight of whites	32.1 g.
Average weight of shell	5.4 g.
Average percent of yolk	30.04
Average percent of white	59.80
Average percent of shell	10.16

The following data, selected chiefly from Asmundson's article cited above, show how small eggs differ from large ones in the quantity and percentage of shell, yolk, and whites:

TABLE IV  
VARIATION IN COMPOSITION OF LARGE AND SMALL EGGS

No. eggs	Average weight per egg	Average weight of			Percentage of		
		Shell	Yolks	Whites	Shell	Yolks	Whites
(Small) 70	49.1 grams	5.0	15.2	28.9	10.3	30.9	58.8
(Large) 70	64.5 grams	6.4	18.0	40.1	10.0	27.9	62.1

On the basis of the edible portion only, small eggs are composed of 34.4% yolk and 65.6% whites; large eggs 31% yolk and 69% whites. The average of the 140 eggs (edible portion) is 32.7% yolk and 67.3% whites. The average percentage of yolk in the edible portion of 885 eggs (large and small) was 33 $\frac{1}{3}$ % and of whites 66 $\frac{2}{3}$ %.

Graham (1908) reports that shell eggs as purchased contain 55.4% white and 31.7% yolk with a white-to-yolk ratio of 1.78.

König (1904) gives the weight and composition of an egg as follows:

TABLE V

Portion	Weight in grams			% of average weight
	Min.	Max.	Av.	
Shell	3.0	7.0	6.0	11.5
White	15.0	43.0	31.0	58.5
Yolk	10.0	23.0	16.0	30.0
Whole egg	32	72	53	100

The edible portion of the average egg contains the following components, in grams:

	Water	Protein	Fat	N-free extract	Ash
31 grams white (albumen)	26.54	3.96	0.07	0.22	0.21
16 grams yolk	8.15	2.57	5.07	0.05	0.16

The following is given as the composition of the ash of egg:

TABLE VI

Portion	Ash, % of dry sub- stance	Percent of the ash								
		K <sub>2</sub> O	Na <sub>2</sub> O	CaO	MgO	Fe <sub>2</sub> O <sub>3</sub>	P <sub>2</sub> O <sub>5</sub>	SO <sub>3</sub>	SiO <sub>2</sub>	Cl
White	4.61	31.41	31.57	2.78	2.79	0.57	4.41	2.12	1.06	28.82
Yolk	2.91	9.29	5.87	13.04	2.13	1.65	65.46	—	0.86	1.95
Whole egg	3.48	17.37	22.87	10.91	1.14	0.39	37.02	0.32	0.31	8.98

König (1904) further states that the shell itself consists of about 89–97% calcium carbonate, 0–2.0% magnesium carbonate, 0.5–5.0% calcium and magnesium phosphates, and 2.0–5.0% moisture and organic matter.

The proximate composition of shell eggs as purchased and on the basis of the edible portion averages approximately as follows (Langworthy, 1901):

TABLE VII

Portion	As purchased	Edible portion
	%	%
Shell	11.2	—
Water	65.5	73.7
Protein	11.9	13.4
Fat	9.3	10.5
Ash	0.9	1.0
Carbohydrates	0.4	0.4
Calories per pound	635.0	720.0

Langworthy also gives the composition of whole eggs (edible portion) and of the yolk and whites as follows:

TABLE VIII

Portion	Water	Proteins	Fat	Ash	Carbo- hydrates	Calories per lb.
	%	%	%	%		
Whole egg	73.7	13.4	10.5	1.0	Trace	720
Yolks	49.5	15.7	33.3	1.1	Trace	1,705
Whites	86.2	12.8	0.2	0.6	Trace	250

According to the same author, there is no appreciable difference in composition between brown eggs and white eggs, nor between eggs from different breeds of hens (provided the hens are fed alike), nor between boiled eggs and fresh eggs, and no great difference between hen's eggs and eggs from other fowl.

Certain markets, *e.g.* New York, prefer white shell eggs; others, *e.g.* Boston, like a brown shell best, but so far as is known there is no appreciable difference in food value between the two kinds of eggs.

Romanoff (1929) points out that fresh eggs have three different layers of albumen, namely (1) outer, (2) middle, and (3) inner, in the relative proportions of 39.8, 57.2, and 3.0, respectively. The percentage of water in the three layers is 88.41, 87.55, 85.45, respectively.

According to Nemetz (1929), the white of an egg is transparent, has a faintly yellow color, and is a ropy fluid with an alkaline reaction. The more jellylike it is, the better. It consists of two parts, a thin fluid and a framework of firm, fibrous material which forms a cellular membrane throughout the mass, thus enclosing the fluid.

The addition of acetic acid or cream of tartar, while whipping, toughens the albumen, making it more elastic and capable of building up a stronger structure.

The solid portion of the white of eggs is almost entirely of a protein nature. The composition of the white, as determined by different workers, shows it to contain from 80.0% to 86.7% water; 10.5% to 12.3% protein; 0.10% to 0.50% glucose; 0.38% to 0.77% nitrogen-free extract; trace of fat; and 0.3% to 0.6% ash. The ash of egg white is composed of 27.7% to 28.5% potash; 32.6% to 32.9% soda; 1.7% to 2.3% lime; 1.6% to 3.2% magnesia; 0.44% to 0.58% iron oxide; 3.2% to 4.8% phosphorus pentoxide; 1.3% to 2.6% sulfur trioxide; trace to 0.10% fluorine; 23.4% to 28.6% chlorine; 9.7% to 11.6% carbon dioxide; and 0.28% to 0.49% silica. Egg white is relatively rich in sulfur.

Sørensen (1934) states that the egg white consists of at least five protein fractions, one of which is a globulin. Each fraction contains a carbohydrate molecule. The percentage of each protein fraction is as follows:

TABLE IX

Protein fraction	% of total protein	Carbohydrate
Globulin	6.7	Contains 4.0% mannose
Mucin	1.9	Contains 14.9% mixture of equal parts mannose and galactose
Albumin	69.7	Contains 1.7% mannose
Conalbumin	9.6	Contains 2.8% mixture of 3 parts mannose and 1 part galactose
Mucoid	12.7	Contains 9.2% mixture of 3 parts mannose and 1 part galactose

The yolk of egg is much more diverse in composition. The percentages of constituents are as follows: moisture 47.2 to 51.8, ash 0.33

to 1.0, fat 20.3 to 22.8, lecithin 7.2 to 10.7, vitellin 15.6 to 15.8, nuclein 1.5, cerebrin 0.3, glycerophosphoric acid 1.2, cholesterolin 0.44 to 1.75, glucose 0.55, and coloring matter 0.5. The principal protein, vitellin, is insoluble in water but soluble in salt solution. It lacks the whipping quality characteristic of the albumen or egg-white. Nemetz (1929) found that it is impossible to whip the yolk to a stiff foam owing not only to the difference in the character of the protein but to the presence of fatty material and the absence of the fibrous structure around which cells can be formed.

The ash of yolk differs quite materially from that of the white. The ash of the yolk is much richer in calcium, phosphorus, and iron and much poorer in potassium, sodium, chlorine, and sulphur.

The following analyses, taken from reports of various workers, show the percentage composition of the ash of yolks: potash 8.1 to 8.9, soda 5.1 to 6.6, lime 12.2 to 12.3, magnesia 2.1, iron oxide 1.2 to 1.5, phosphorus pentoxide 63.8 to 66.7, fluorine trace to 0.8, chlorine trace to 1.9, and silica 0.55 to 1.40.

König (1904) reports that 100 g. egg yolk contains 1.279 g. phosphoric acid, of which 0.478 g. is in the form of lecithin soluble in ether (58% of the total), 0.345 g. as lecithin insoluble in ether (42% of the total), 0.178 g. as nuclein, and 0.278 g. as glycerol and inorganic phosphates. The phosphoric acid in the protein of the yolk, soluble in boiling alcohol, amounts to 0.823 g., equivalent to 9.35% of distearyl lecithin.

The yolk contains practically all of the fat of the egg. This fat is present in a finely emulsified state, some of it being combined with lecithin, a phosphorus-containing fat-like substance present in egg yolk to the extent of 7% to 10%. Lecithin, besides containing compounds of phosphorus, is an important source of iron and a good source of calcium, sodium, potassium, and magnesium. It is relatively rich in vitamins A, B, and D, and contains some E. The yolk is an important source of growth-stimulating phosphorized fat, the latter being composed largely of palmitin, stearin, and olein. The iodine number of yolk fat is 68.5; that of the fatty acids is 72.6. The melting point of the fatty acids is 36°C.

### Freezing

The process of freezing eggs without the use of preservatives is of American origin. In this country, before 1930, freezing was confined almost exclusively to whole eggs. In 1937, however, 29.5% of the frozen eggs produced in the United States was whites, 26.5% yolks, and 44% whole eggs or mixed whites and yolks. Since 1922, the quality of the eggs used for freezing has improved considerably and in

plants located in the surplus-egg-production regions eggs for freezing now consist largely of "current receipts" or "shippers' firsts" (U. S. Tariff Com., 1929). According to this same authority, liquid or chilled eggs, including whole eggs, whites, and yolks (so-called "intermediate products"), are made in large quantities in this country from "checks" or "cracked" eggs. These, however, are used chiefly for industrial purposes.

Formerly, China produced large quantities of liquid yolks, preserved with boric acid, and exported the product to the United States for use in the arts. The United States no longer imports such yolks in any appreciable quantities.

Frozen eggs are eggs which are broken, frozen, and kept frozen until used. In high-grade establishments no bad eggs are ever used. Every precaution is taken to prevent contamination. The utensils are sterilized and the cans of broken eggs are removed to the freezer immediately.

A. K. Epstein (1931) is of the opinion that when eggs are preserved at 30° to 32°F., or at the usual freezing temperature, certain changes take place which affect the physical and colloidal properties of the eggs and their function in cake making, for the emulsifying, lubricating, and gas retention properties are in various degrees impaired. To overcome these defects egg yolks are processed with sugar before freezing (Keith did this in 1912). Glycerin is now used for the same purpose. Also a combination of carbohydrates, acids, and salts is added to the egg yolk to prevent deterioration.

In commercial practice eggs are frozen at a temperature of from 10°F. to -10°F. This procedure prevents the growth of bacteria, one of the main causes of spoilage. Eggs when frozen and maintained in that condition undergo no decomposition due to microorganisms and such eggs may be kept for years without loss of vitamin A.

Certain complex physical changes do, however, take place in the egg colloids, even in eggs frozen in the manner described above, and especially while the egg-mass is in the process of freezing and during subsequent thawing. For example, when egg white is frozen and thawed it separates into fluid and viscous portions. If frozen gradually, ice crystals will form and when thawed the melted ice crystals will not be reabsorbed by the coagulated portion of the egg white. If, however, the egg white is frozen very quickly the water will be reabsorbed after thawing and the egg white will revert to its original physical condition. The lower the temperature of freezing, the greater will be the quantity of the thin egg white. The thin portion of fresh egg white is 42% of the total; if the white is frozen to a temperature of

3°F. this portion may become as high as 65% to 67%. If the frozen white is kept for four to five months, a coagulation of certain protein material of the albumen takes place and on thawing white fibers of mucin (not the chalaza) will be visible in the viscous portion of the egg whites.

Again Epstein (1931) states that when the yolk is frozen, *e.g.* at 23°F. ( $-5^{\circ}\text{C.}$ ), a solid mass will be produced and on thawing the product will revert to the original fluid and homogeneous state without change. If, however, the temperature of freezing is lowered, which must be done to prevent biochemical changes, the yolk undergoes a more radical change from a physical-chemical standpoint. In this case the yolk does not revert to the original fluid state but becomes a gummy, rubbery mass, depending upon the length of time in storage. The volume of the thawed product will be less than that of the original material and the lecithin will have become chemically altered, in that on long storage it decomposes with the splitting off of free phosphoric acid. As the freezing progresses, ice crystals separate, bringing about an appreciable concentration of the salt of the yolk, *i.e.* from a normal 1% to 10% of the non-frozen liquid. At 21°F. the lecithin is combined with or dissolved by the 10% salt solution and later precipitated and thrown out as flakes.

Modern facilities for freezing include the following (taken largely from Ovson, 1938):

- (1) A refrigerating room for the storage of shell eggs, where they are chilled for several days at 31°F. This makes the whites stiffer and more gelatinous and facilitates the separation of whites and yolks.

- (2) A candling room kept at 10° to 13°C. (50° to 55°F.). Here the eggs are selected according to size, quality, and shell texture, all inferior eggs being eliminated.

- (3) A breaking room whose temperature is maintained at 16° to 18°C. (61° to 64°F.). The eggs, on an endless chain, are received from the candling room and broken by means of a sharp-edged copper, aluminum, or stainless steel blade; each egg is broken and tested and inspected for odor and appearance. Dirty eggs are broken separately; all eggs unfit for food are sold to tanners. When desired the whites and yolks are separated.

- (4) Churns, used to break the yolks and to mix them with the whites preparatory to freezing. The churns are used also to mix such ingredients as salt, sugar or glycerin with the egg yolks. The sugar and glycerin are added in order to prevent the formation of lumpy particles of yolk and to permit of smooth thawing. The churns are equipped with strainers for the removal of shell and other foreign matter.

(5) Egg white strainers, used in removing shell particles, chalaza and other interfering foreign materials, leaving the clear product capable of rendering the maximum beating volume.

(6) Refractometer room, to determine solids from the refractometric index.

(7) Sterilizing room, where all utensils and apparatus may be sterilized.

(8) Sharp-freezing room where the eggs are kept at  $-10^{\circ}\text{F.}$  to  $-15^{\circ}\text{F.}$  for 72 hours.

(9) Storage freezer room, temperature  $0^{\circ}\text{F.}$  or lower.

(10) Dry storage space for empty cans and cases.

All rooms are, or should be, built with rounded angles and with walls of tile. To freeze eggs with such equipment requires about eight minutes from the time the egg is candled.

Chinese factories for the freezing of eggs are, according to Bordas (1922), of English or American origin.

There is practically no difference between the freezing methods used in China and in the United States. In both countries the eggs are chilled, candled, broken, and whites and yolks separated if desired; contents of broken eggs, in a cup, are tested by smelling (to eliminate any musty eggs, for one such egg will spoil the whole egg-mass to which it is added). Whole eggs and yolks (but not the whites) are churned before freezing in order to thoroughly break up the chalaza and the yolk membrane, which are more or less objectionable in baking, and the frozen eggs are kept at a low temperature until shipped (U. S. Tariff Com., 1929).

In recent years important improvements have been made in egg freezing methods. The older method, freezing at  $0^{\circ}\text{F.}$  or below, required 72 to 100 hours to freeze a can (30 pounds or more) of liquid eggs. During this slow freezing process some physical and bacteriological changes took place. Large ice crystals were formed, indicating that the protein (especially of the yolk) had been deprived of some of its water and had become partially coagulated, thus lowering the fluidity-and-solubility factors and, at the same time, lowering the whipping-and-baking properties of the egg. The addition of certain protective substances, *e.g.* gelatin, dextrin, glucose, lactose or glycerin, before freezing minimizes the formation of large ice crystals and to a certain extent reduces the dehydrating action of slow freezing. These advantages are, however, claimed for the so-called "quick freezing method" now being used (Swift's Frozen Eggs).

The quick method of freezing eggs depends upon the preparation of a homogeneous emulsion of eggs by disintegrating the chalaza, the

germ, and the membranes and dispersing them into the egg liquid. This homogeneous emulsion is then fed in a thin film to a mechanically chilled refrigerated roll which brings the liquid egg into a frozen condition in about seven seconds instead of ten times as many hours. The frozen mass is then packed into sterilized antirust cans which are immediately transferred to a sharp sub-zero freezing room. This short-time freezing prevents an increase in bacterial count and is claimed to yield a product with a smooth, uniform texture free from large ice crystals. Egg whites, whole eggs, and egg yolks may all be frozen by quick freezing processes. In 1937, out of 225 million pounds of frozen eggs or egg products, some 29% was frozen whites, about 36% frozen whole eggs, nearly 27% frozen yolks, and 8% mixed eggs. The statement is made by Sharp (1937) that whites are not affected by freezing but that yolks are affected and hence the whole egg undergoes certain changes.

There is little available information regarding the effect of freezing upon the bacterial or microbial flora of eggs.

As the result of freezing, the eggs expand in volume; hence it is well not to fill the cans quite full before freezing. After freezing the contents, the cans are filled to the top and again frozen, after which the temperature of the eggs is maintained at 0° to -5°F. until they are sold.

Eggs stored at a temperature of -0.5° to 0.5°C. keep perfectly well for months. This temperature (-0.5°C.) is very close to the congealing point of liquid egg. Fresh whole eggs congeal at -0.44°C., the whites at -0.54°C. (Monvoisin, 1928).

In frozen eggs six to seven months old, the yolk contains 5.3 to 11.4 mg. of ammonia per 100 g. of yolk. In fresh egg yolk the quantity of ammonia is only 1.7 to 3.9 mg. It has been found that clean eggs keep better than dirty eggs after freezing, the latter containing, after two months in the frozen state, 7.2 mg. of ammonia as compared to 3.9 for the former.

C. Bidault (1928) noted that eggs kept in cold storage developed ammonia and that at the end of six months the quantity of ammonia nitrogen approached but did not exceed 5 mg. per 100 g. of yolk. Eggs that contain as much as 5 mg. of ammonia nitrogen per 100 g. are no longer fit for use as eggs boiled in the shell.

### Composition of Frozen Eggs

Redfield (1920) shows that frozen eggs have the following composition:



TABLE X  
COMPOSITION OF FROZEN EGGS

	Solids	Fat	Ammonia nitrogen	Acidity of fat	Reducing sugars
	%	%	%	%	%
Frozen eggs	27.1-29.1	10.6-11.5	1.9-2.4	1.84-1.92	0.27-0.31
Eggs held in cold storage 4 to 8 months	27.9-29.2	10.5-10.8	2.6-2.7	1.50-1.83	0.31-0.36
Fresh eggs—19 samples	26.0-27.2	9.3-11.0	1.0-2.0	1.21-1.72	0.29-0.36
Frozen yolk	43.2-43.4	23.0-23.3	4.1-4.4	1.80-1.81	0.18-0.20
Yolk held in cold storage 4 to 8 months	39.4-43.1	19.2-22.1	3.8-4.9	1.53-1.82	0.18-0.23
Fresh yolk	46.9-50.9	24.5-28.8	2.5-3.1	1.44-1.96	0.08-0.20
Frozen whites	—	—	—	—	—
Whites held in cold storage 4 to 8 months	14.0-14.6	0.03-0.07	0.4-0.8	—	0.37-0.48
Fresh whites	11.8-12.8	0.01-0.12	0.1-1.2	—	0.31-0.46

Swift and Company report that frozen whole eggs, frozen whites, and frozen yolks have the following composition:

TABLE XI  
COMPOSITION OF FROZEN WHITES AND YOLKS

	Water	Protein (N × 6.25)	Fat	Ash
	%	%	%	%
Whole	73.7	13.3	12.3	0.7
White	87.2	12.0	0.2	0.6
Yolk	56.7	15.2	27.1	1.0

When egg yolks are frozen at  $-6^{\circ}\text{F}$ . or above they remain unchanged after thawing. If frozen at below  $-6^{\circ}\text{F}$ . the yolk on thawing changes to a non-fluid condition. Quick-frozen eggs possess a uniform, homogeneous consistency at all times.

When properly stored, frozen eggs undergo but little physical, chemical, or bacteriological changes.

### Methods of Thawing or Defrosting

Defrosting should be done by placing the cans of frozen eggs in a vat of running water. In five to six hours the eggs will be defrosted and ready for use. Defrosting the eggs near the oven subjects that portion of the eggs near the outside of the can to a temperature of  $55^{\circ}$  to  $60^{\circ}\text{F}$ . for 15 or more hours as compared to a temperature of  $33^{\circ}$  to  $34^{\circ}\text{F}$ . for 5 to 6 hours when defrosting is done in running cold water. In the former case the eggs may become curdled; in the latter, they remain relatively smooth in appearance, retain their fresh flavor, and produce cakes of superior quality.

Bakers should not have on hand more frozen eggs than they need for current use. Frozen eggs, once thawed or defrosted, are very perishable and should be stored, until ready for use, at that temperature which causes no unfavorable change (Swift and Company).

Great care should be exercised in thawing out the whites. These should be kept in an ice box until thoroughly thawed, after which they should be mixed. The surface of the can should never be exposed to high heat as this will coagulate the albumen. During the freezing of the whites the solids are concentrated in the center of the can; hence the entire can should be thawed and thoroughly mixed before use (Armour and Company, 1929).

F. Bordas (1922) suggests that the baker should stipulate that all containers of frozen eggs, when received, should show evidence that they have not been previously opened and with no signs of leakage or sweating. A guarantee should also be required that the contents have not been thawed.

### Characteristics of Frozen Eggs

Frozen eggs behave in baking very much like shell eggs, and can be used wherever shell eggs are used. Eggs exert a binding action when used in cake making; they are capable of leavening five to six times their weight of other ingredients, thus retaining the air which has been beaten into the mix; they have a considerable emulsifying power; due to their fat content ( $\frac{1}{4}$  of the yolk is fat) they have a shortening action; they improve the flavor of the product (no substitute has been yet found that will give the flavor produced by eggs). Furthermore, eggs give the product a more pleasing color. They enhance the cell structure of the product, maintaining it during the baking process, and restrict the vaporization of moisture from the baked product, thus keeping the product fresh for a longer period. All of this adds greatly to the appetite appeal. Eggs add appreciably to the food value of the product, as eggs are rich in protein, fat, lecithin, minerals and vitamins.

Frozen eggs are more convenient to handle than are shell eggs. After thawing they can be easily ladled from the container. They are also more uniform in quality than shell eggs and in general better adapted to large-scale usage. Wholesale bakers and confectioners use mainly frozen eggs in manufacturing their products. With the use of frozen eggs there is no waste (some 3% to 4% of the whites may be lost in the breaking process), they require less space for storage, and there are no transportation charges for shell and for bad eggs. Frozen eggs are cheaper to use the year around than shell eggs.

Frozen eggs retain their original quality almost indefinitely if kept

frozen. The yolks are firmer and the whites thicker than are the corresponding portions of stored shell eggs. Frozen eggs should have an ammonia-nitrogen content not to exceed 0.002%, frozen yolks 0.003%, and frozen whites 0.0004%.

Frozen whole eggs (sometimes mixed with frozen yolks) are used in doughnut mixes, cake mixes, sweet doughs, for most kinds of cakes, and also for jelly bases, cookies, and pastries. Bakers generally use more frozen whole egg than frozen yolks. Frozen whole eggs can be used even in making omelets of very good quality.

Frozen eggs are gradually replacing the fresh shell eggs and storage eggs in the preparation of many important commercial food products.

The principal uses for frozen eggs—whole, yolks, and whites—are in the baking of cake, in the manufacture of noodles and candies, and in the preparation of mayonnaise, salad dressings, icings, ice cream, food beverages, and certain medicinals. In 1935 over 24 million dollars worth of eggs (fresh, frozen, and dried) were used by the baking industry alone. Table XII shows at a glance where the various frozen egg products are used. This information is taken largely from a recent article by Leo D. Ovson (1938).

Frozen eggs have almost entirely replaced dried eggs in those products in which either might be used. Frozen yolks are useful in sweet goods, for making doughnuts, and in the manufacture of noodles, mayonnaise, salad dressings, etc. Yolks have a high food value and greatly improve the color of the product manufactured. Yolks are half water, one-third fat, and one-sixth protein, and are a source of lecithin and vitamins. While yolks cannot be whipped, as can the whites, they still can carry a certain amount of air and do leaven to a certain extent, especially when sugar has been mixed with them. The addition of sugar to the liquid eggs before freezing improves the consistency of the thawed eggs after freezing and makes them more nearly like fresh eggs.

Egg yolks are used in gold cake or other cakes where a deep yellow color is desired (the color of the yolk is largely dependent upon the character of the food consumed by the hen). Yolks are also used in connection with whole eggs in doughnut mixes, cake mixes, or wherever a deeper color or greater emulsifying power is wanted. A sponge cake made with egg yolks will have finer cell walls and will hold its cell structure during baking better than an angel food made from egg whites. This is because of the greater emulsifying and stabilizing properties of the egg yolk. It is claimed that cakes baked with glycerin-yolk (yolks plus 5% pure glycerin) remain fresh longer than those made without the glycerin.

Frozen egg whites are (like fresh egg whites) about six-sevenths water and one-seventh protein (albumen). They lend very little flavor to the baked product, but give a mellowness to the finished cake. The peculiar structure of the egg white furnishes thin but strong walls for the tiny air cells formed when egg white is whipped. Even a small quantity of grease, fat, or of egg yolk injures the whipping quality of egg whites. Egg whites properly prepared have excellent solubility.

Frozen whites are used in making angel food, white pound cake, certain kinds of layer cake, box cakes, fruit cake, loaf cake, cup cake, cheese cake, etc., cream icings, cooked marshmallows, meringues, macaroons, and confectionery; also for white sponge base, apple sauce cake, custard pie, white fruit pound cake, ice box cookies, etc.

Based upon laboratory studies over 65% of the cost of ingredients used in making angel food is for egg white; for making gold cake and pound cake the cost of yolk and of whole egg is over 40% (in each case) of the total cost for ingredients. The monetary value of eggs used in cake making is over  $2\frac{1}{2}$  times that of shortening. Of the total quantity of ingredients used in making angel food 40% is egg white; in the making of gold cake 20% of the weight of the ingredients is yolk; for pound cake 25% is whole egg. On the basis of the flour used in cake making, egg white in angel food is 225%, yolk in gold cake 83%, whole egg in pound cake 100%. The importance of eggs in cake making should not be belittled.

For angel food cake, fresh or frozen egg whites are superior in foaming qualities to the average commercial dried egg white, when whipped with cream of tartar or calcium acid phosphate, or other acid ingredients. One very important point noted in the use of egg white, both from fresh eggs and from eggs that had been stored for nine months, was that the thin portion made a better cake than the more viscous albumen. The thin portion of egg white can be whipped more readily to the proper consistency. Apparently air is more easily incorporated into the thin or less viscous whites. It was further noted that when enough water to make the viscosity comparable to that of thin egg white was added to the thick or viscous portion of the whites an improved angel food cake was obtained. It is of the utmost importance, therefore, that in all investigations in which egg white is used, either a separation be made of the thin and thick whites and each used separately or that a uniform mixture of the two kinds of whites be made. These results confirm those obtained by Hunt and St. John (1931), who demonstrated that adding water to the thick portion of egg white improves its whipping qualities.

Our experiments have shown that as much as a third of the egg

white in an angel food cake recipe can be replaced by water without affecting appreciably the quality of the cake. Increasing the egg white by one-third, however, did not result in a satisfactory cake from the standpoint of appearance.

In making pound cake, increasing the amount of egg in the laboratory formula by one-third to one-half somewhat improved the grain and texture, but not the size or general appearance. Decreasing the amount of egg by even one-third made the cake soggy or "sad," with a smaller volume and poorer grain, texture, and general appearance. If the amount of egg is decreased, as it is in many commercial pound cakes, it is necessary to increase somewhat the amount of both fat and milk to make a satisfactory product (Bailey and LeClerc, 1935).

Both fresh and frozen eggs make satisfactory cakes of all types. Occasionally frozen eggs produce cake of larger volume than do shell eggs, probably because of the greater uniformity of quality inherent in frozen eggs.

Cake volume is controlled largely by the use of eggs. Sponge cake and cream puffs made from eggs laid and broken in the spring will produce greater volume than if made from eggs laid and broken during the summer months. Experimental cakes were made with eggs produced in April, July, and September. The grading of these cakes showed that those made with April eggs were 15% and those made with September eggs 10% larger than the cakes made with July eggs. Cream puffs made with April eggs were also larger and smoother than those made with July and September eggs (Nemetz, 1929).

TABLE XII  
THE USE OF FROZEN EGG PRODUCTS

Food product	Whole or mixed	Whites	Plain yolks	Salt yolks	Sugar yolks	Glycerin yolks
Biscuits and cookies	X	X	X		X	X
Dark cake and doughnuts	X		X		X	X
White cake, meringues, marshmallows, confections and candies		X				
Custards			X		X	
Noodles	X		X			
Food beverages	X	X			X	
Ice cream	X	X	X		X	
Mayonnaise			X	X	X	X
Salad dressings	X		X	X	X	X
Medicines		X	X			
Pie and icings	X	X				

A small quantity of frozen whites is used commercially in the making of prepared puddings. No frozen egg products (at least no appreciable quantity) are used in the home or by restaurants.

Frozen yolks produced gold cake only slightly inferior in volume, grain, and appearance to that made with fresh yolks. The same was found true in the case of pound cake. Frozen whites made better cake than the dried whites which had been reconstituted 7 : 1. Likewise, the frozen whole egg produced better sponge cake than did the dry egg yolk and dry whites, both reconstituted.

### Mayonnaise

Mayonnaise was first made commercially in the United States in 1906. Its growth as a commercial product has been rapid. Statistics show that in 1937 more than 9 million gallons of mayonnaise were made by 71 companies, representing 80% of the industry.

If we take  $7\frac{3}{4}$  pounds as the average weight of a gallon of mayonnaise, the weight of these 9 million gallons would amount to 70 million pounds. Since mayonnaise contains approximately 10% of egg yolk, there would be required 7 million pounds of yolk to be obtained from 197 million eggs or there would be required 548,000 cases of eggs to produce these 9 million gallons of mayonnaise.

In addition to mayonnaise there are salad dressings and related products on the market that have egg yolk as one of their constituents. While the percentage of egg yolk in salad dressings is variable and always less than in mayonnaise, large quantities of yolk are used in this connection for there is twice as much salad dressing produced as there is mayonnaise.

An analysis of ten brands of commercial mayonnaise (Epstein, Reynolds, and Harris, 1937) shows that they contain from 77% to 83% of oil, 10% to 15% of total moisture, from 0.3% to 0.5% of acid as acetic acid and from 7% to 11% of commercial egg yolk.

Most manufacturers use egg yolks, but there are a few who use whole eggs. Frozen yolks are the type generally used in making the mayonnaise. Dried yolks, either spray-dried or flaked, are not used by the mayonnaise manufacturers, for the yolk-oil becomes rancid in the dried product and it will have a tendency not only to reduce the biologic value of the mayonnaise from the vitamin standpoint but to induce rancidity in the mayonnaise itself.

Within recent years a number of special frozen egg products have been prepared to meet the requirements of the mayonnaise manufacturer. Such preparations are made from selected fresh eggs, separated in sanitary plants under sanitary conditions and treated by special processes, so as to prevent the multiplication of microorganisms during the time the product is allowed to thaw out in the mayonnaise plant and also to enhance the emulsifying properties of the yolk.

### **Candy, Confections, Ice Cream, etc.**

While egg yolks are generally used by the mayonnaise manufacturer, egg white or albumen is used mostly by the candy manufacturer and the confectioner. Many candy manufacturers prefer egg albumen to fresh egg whites. Egg albumen was formerly imported from China but now much of it is produced in the states of Oregon, New York, and California.

One candy maker in Chicago uses in his plant 180,000 fresh eggs daily; this amounts to 500 cases of eggs for this one manufacturer and there are many manufacturers throughout the country.

### **Dried Eggs**

Dried eggs are prepared in flake-powdered or granular forms, the quality of the products depending largely upon the manner of drying and the temperature at which the eggs are dried. Too high a temperature during drying will harden and coagulate the albumen, thus decreasing its solubility as well as its leavening power.

The drying of eggs began in Europe in the sixth decade of the nineteenth century (Sudendorf and Penndorf, 1924). The first experiments were carried on near the Russian-Austrian border, the object being to lessen the losses due to spoilage of shell eggs. Factories for the drying of eggs were erected in that part of Europe toward the end of the same century. The eggs were dried over direct fire in tin or zinc plates, the resultant product when ground consisting of a horn-like powder. This product was used, not as a food, but almost exclusively by such industries as leather, dye, photographic, and paper. Drying of eggs in China was started in the early years of the twentieth century. At first and for some time, such preservatives as boric acid, benzoic acid, salicylic acid, fluorides, etc., were used by manufacturers of these products. The early processes for drying eggs were necessarily primitive, very slow and yielded a rather unsatisfactory product. The product decomposed during drying unless a preservative was used.

In the early days of egg drying, good eggs were separated from the bad by candling or difference in specific gravity by the use of a 6% to 8% salt solution. The bad eggs, being lighter, floated in the salt solution.

Drying of eggs eliminates much of the weight as water and thus brings about a great saving in transportation cost, as well as in storage space. Fresh shell eggs contain about 80% water. In the process of drying, 90% of the water is driven off. Spray-dried whole eggs and egg yolk contain about 3½% moisture; flake eggs contain from 7% to 12% moisture. The liquid-egg equivalents necessary to make one

pound of the dried egg product are approximately 2.23 pounds of yolk, 3.56 pounds of whole egg, and 7.30 pounds of liquid whites (U. S. Tariff Com., 1929).

Since the advent of the frozen egg industry the output of dried eggs has been insignificant when compared to that of the frozen product. Commercial egg drying in the United States practically ceased in 1916 except when the price of eggs was extremely low. From 1916 to 1931 there was little domestic drying of eggs in spite of the high tariff of 1922 (U. S. Tariff Com., 1931). Until 1927 eggs were dried in the United States only experimentally, but in that year some commercial drying of whole eggs and of egg yolks was undertaken, largely owing to the shortage of dried eggs in China. No eggs were dried in this country in 1928, and only small amounts in 1930 and 1931 because manufacturing costs were prohibitive. Chinese egg-drying plants, located chiefly in the Yangtze Valley west of Shanghai and as far as Hankow, are owned generally by foreigners, mostly American and British, and most of the domestic consumption of dried eggs has been the imported products from China (U. S. Tariff Com., 1929). During 1928 to 1930 the average imports of dried eggs (chiefly from China) were equivalent to 1.6% of the amount of shell eggs and half the quantity of frozen eggs produced in the United States (U. S. Tariff Com., 1931).

Dried egg whites form the bulk of the imports of dried egg products; dried yolks are of secondary importance. Only a relatively small quantity of dried whole egg is obtained from overseas. The principal trading in dried eggs in this country is in New York City and they are distributed from that point.

Until recently China was the only producer of dried eggs. According to the U. S. Tariff Commission (1929), the exports of dried eggs from China during 1924-26 were as follows:

TABLE XIII

Product	1924	1925	1926	Destination
	<i>1000 lbs.</i>	<i>1000 lbs.</i>	<i>1000 lbs.</i>	
Dried albumen	8,796	8,694	7,800	(80% to Gt. B. and U. S.)
Dried yolks	8,768	11,225	8,585	(75% to U. S. and Ger.)
Dried whole eggs	3,068	11,293	2,912	(66% to Gt. B.)

During 1928 to 1930 the imports of dried eggs, calculated to the equivalent of shell eggs, amounted to 35 million dozens or 1.5% of the combined domestic consumption of shell, frozen, and dried eggs. The imports of dried eggs into this country, a few years ago, were approximately as follows: 5% in the form of whole egg (entirely



sprayed), 20% albumen (practically all flaked), and 75% yolk (almost entirely sprayed).

During 1930 to 1931 the cost in this country of spray-drying of whole eggs was 3.9 cents per pound; spray drying of yolks cost 3.3 cents, and tray-drying of whites, 11.2 cents. In the United States, the cost of drying eggs is about one-tenth the cost of the shell eggs (U. S. Tariff Com., 1931).

According to the same authority, the cost of domestic dried egg products delivered at New York City was as follows:

TABLE XIV

Raw materials	Cost prices—cents per lb.		
	Whole egg	Yolks	Whites
(1) Cost, liquid whole egg at plant	22.7	—	—
(2) Ratio of value of yolk and whites to whole egg	100.0	146.1	56.5
(3) Value at plant	22.7	33.2	12.8
(4) Drying ratio, pounds of liquid material per pound of dry product	3.65	2.23	7.30
(5) Cost of liquid raw material per pound of dried product, (3) × (4)	82.9	74.0	93.4
(6) Drying costs	3.9	3.3	11.2
(7) Containers and packing	1.1	1.1	1.1
(8) Total cost at plant of dried egg product, (5) + (6) + (7)	87.9	78.4	105.7
(9) Transportation to New York City	1.6	1.6	1.6
(10) Cost delivered at New York City	89.5	80.0	107.3

There are three grades of dried yolk, each having a different commercial value. The flake is the most expensive, the spray powder next, and the granular product the least per unit. The domestic spray-yolk commanded in 1936 a higher price (68 cents) per pound than did the imported flake yolk (57 cents) on the average for the year. At the same time the domestic albumen sold for \$1.08 per pound as against 80 cents for the imported flake albumen. In the case of whole egg there are only two grades; the flaked again is sold at a higher price than the sprayed product. In general, the flaked product is more soluble than the sprayed, easier to handle, and therefore commands a better price. There is no so-called "granular whole egg product."

Liquid yolks are valued at 146.1% of the value of liquid whole egg, and liquid whites at 56.5%. Hence when liquid whole eggs are at 22.7 cents per pound (equivalent to about 27 cents per dozen for shell eggs) liquid yolk should be 33.2 cents and whites 12.8 cents per pound.

Armour's Research Bulletin (1929) states that on a day when eggs, by the case, were selling at 33 cents per dozen, frozen egg whites were

selling at wholesale for 17 cents per pound. On this basis a case of whole eggs would cost \$9.90 and the same weight of frozen whites, \$7.31. Hence egg whites are occasionally more economical than are whole eggs.

Following the primitive system of drying in pans came the belt method (now practically discarded), the vacuum method, and later the spray drying method, the latter being used most extensively in the case of whole eggs and yolks, especially the latter. Only for the drying of egg whites is the pan method mostly used, since it has been impractical up to the present to use the spray system for the whites. According to Winckel (1925) vacuum-immersion, steam-heated rolls are largely used in Germany for drying whole eggs. By this method the eggs are dried in 8 to 10 seconds and at a sufficiently low temperature that coagulation of the whites does not take place. The absence of air in this method prevents bacterial contamination, oxidation, and rancidity; this method in this respect is superior to the spray process. Spray-dried eggs are, however, claimed to be superior in emulsifying power and in solubility (Sudendorf and Penndorf, 1924).

### Methods of Drying

In this country, eggs are dried by any one of three general methods, spray, belt, and tray or pan. Up to and including the breaking stage, the preparation of eggs for drying is identical with that followed for freezing.

*Spray method* (Swenson, 1938).—After breaking, the liquid whole eggs or yolks are pressure-sprayed into the upper part of a high-ceilinged chamber heated to 160°F. The product is collected from the lower part of the chamber as a fine powder containing 3% to 8% moisture. The dried powder then usually goes through a sieving process; it is finally collected in bins and packed in sealed tin-lined wooden cases of 200 pounds net weight or in paper-lined barrels. The machinery employed in this process has been adapted from that used in milk-drying plants. Many plants can shift from milk-drying to egg-drying on relatively short notice, but the low price of imported dried eggs makes it impracticable quite often to dry eggs in the United States. Egg yolks are dried chiefly by this process. The whites are generally dried in pans or trays.

*Belt method*.—This method (now very little used) is, as the name implies, simply a process of allowing a thin film of liquid egg to flow onto an endless belt. Drums or discs can also be used as well as belts. The belt passes through heated chambers through which heated, filtered air circulates at about 135° to 140°F. The belt, which should be made

of non-corrosive metal, is of sufficient length and the temperature is so controlled that the egg film is dried in one revolution, after which the dried product is automatically scraped off by means of a metal scraper and allowed to fall into drawers or bins. The first drying requires from one and one-half to two hours. The product is next spread on wire screens and further dried by placing in a "finisher," a large cabinet kept at 100° to 110°F., where it is held for two to three hours, after which it may be graded into flakes of different sizes or powdered. The finished product usually contains from 3% to 8% moisture. Some yolks are dried by this process, the product being somewhat more soluble than that produced by the spray method. In drying the whole egg every attempt should be made to keep the temperature no higher than 130°F. in order to avoid coagulation of the albumen. This method has fallen into disuse and is not a generally accepted method at the present time.

• *Tray or pan method.*—This method, which is in general commercial use, is carried out by spreading the liquid egg in suitable pans or trays made of aluminum or some of its alloys. Use of other metals is avoided because they react with the egg protein to discolor and partially denature it. The trays are placed on shelves in especially constructed cabinets through which a forced draft of hot air at 110° to 120°F. (albumen coagulates at 126°F.) is circulated, carrying the moisture out through appropriate ducts. Drying of egg white is usually completed in 18 to 24 hours. Egg albumen cannot be spray-dried economically, the temperature being too high for the delicate character of the albumen and the whipping quality of the product being thereby seriously impaired.

Attention should be called to the two types of dried egg albumen now being manufactured. They are the unfermented and fermented products, which are characterized by the method employed for thinning the albumen prior to drying.

*Unfermented albumen.*—Unfermented thin white may be obtained in several ways. The two most common methods are mechanical pumping through mixing pumps and acid hydrolysis. The latter, however, is controlled by patents. Still another method, developed by the U. S. Department of Agriculture, is the use of protein-splitting enzymes, such as trypsin. These methods all yield thin white within comparatively short periods of time and little or no foam is formed. The enzyme process requires about 36 hours and is the nearest approach to a quick, natural change of any of the methods discussed.

*Fermented albumen.*—This is egg white which has been allowed to stand in large open vats or tanks at room temperature for a sufficient

length of time to allow the naturally occurring enzymes, and also innumerable proteolytic microorganisms, to completely liquefy the albumen. This usually requires from 4 to 6 days. The white, at this point, has a heavy, odorous, spongy foam or scum over the surface. The liquid white, under the scum, has a watery consistency, an odor comparable to that of alfalfa hay, and a slightly salty taste. It is acid in reaction (pH 5.5) and is neutralized with ammonium hydroxide prior to drying.

In China, egg whites are usually dried on belts or drums after fermentation has taken place and after the acidity has been neutralized with ammonia.

*Recovery of the foam.*—Albuminous egg-foam, as it comes from the fermenting vats, has an acid reaction of approximately pH 5.0. Liquefaction of this material may be accomplished as follows:

To 50 gallons of foam add about two quarts of 20% citric acid solution, stirring constantly and mixing thoroughly. Dissolve 5 g. of commercial dried pepsin in about one pint of citric acid solution and stir into the acidified foam. When liquefaction is complete neutralize with ammonium hydroxide and dry in the usual manner.

Dried foam, when reconstituted, gives an excellent whip, yielding a firm-bodied meringue. When tested in icings and cakes it gave the same result as any fermented albumen (Swenson, 1938).

A recent patent issued to Tranin (1938) describes a process which, it is claimed, will make an improved dry albumen. The egg white is mixed with glucose in sufficient quantity to act as a preservative during the dehydration and to prevent the whites from becoming sour when later  $\frac{1}{2}\%$  of tartaric or lactic acid has been added. The egg white-glucose mixture is heated to 75°F. to reduce the viscosity of the mixture,  $\frac{1}{2}\%$  of the acid in solution is mixed in with the liquid egg white, and the mixture is allowed to stand for 4 to 30 hours. The reaction effects separation of the objectionable chalaza and stringy materials which rise to the top to form a scum. After this scum is removed the purified whites are then dehydrated in shallow pans, or spray-dried.

### Reconstitution

Dried egg products are easily reconstituted by addition of water, as follows:

*Dried whole egg.*—In practice use one part dried egg to three parts of water. Allow to stand four to five hours or until normal liquid egg consistency is obtained.

*Dried yolk.*—Theoretically the ratio of one part of yolk and one part of water should be used, but practically two to three parts water are required for one part of yolk. Allow to stand one hour.

*Unfermented albumen.*—One part dried albumen to six or seven parts of water will produce a product very similar to fresh whites. Allow to stand three hours.

*Fermented albumen.*—Use one part of dried albumen to 10 parts of water. Allow to stand three hours.

All reconstituted egg products should be used as soon as possible since they are comparable to fresh eggs and are, therefore, very perishable. One pound of dried egg product is produced from  $3\frac{1}{2}$  pounds of whole egg, or  $2\frac{1}{4}$  pounds of yolk, or  $7\frac{1}{4}$  pounds of whites (Anon., 1932).

TABLE XV  
THE COMPOSITION OF DRIED EGGS AND OF FRESH EGGS  
(Average from Various Sources)

	Water	Protein (N×6.25)	Fat	Carbo- hydrate	Ash	Calories per pound
	%	%	%	%	%	
Shell eggs as purchased	65.2	11.8	11.0	—	0.6	665
Shell eggs—edible portion:						
Whole	73.2	13.2	12.0	—	0.7	720
Yolk	49.5	15.7	33.3	0.4	1.1	1,704
Whites	86.2	12.3	0.2	0.7	0.6	250
Dried whole egg	6.4	46.9	36.0	7.1	3.6	2,525
Dried yolk	5.9	33.3	51.6	5.7	3.5	2,794
Dried whites	11.7	73.2	3.3	5.6	6.2	1,501

TABLE XVI  
EGG ANALYSES, AIR-DRY BASIS<sup>1</sup>

	Water	Protein (N×6.25)	Alcohol ppt. nitrogen	Total nitrogen	Lipoids	Lipoid- phosphoric acid
Whole egg (8 samples)	5.5– 9.3	43.8–46.3	2.8–3.3	7.0– 7.5	42.0–48.8	1.2–1.4
Yolk (5 samples)	2.9– 4.7	32.6–33.4	0.5–0.8	5.1– 5.4	62.8–64.0	1.6–1.7
Whites (6 samples)	11.7–16.2	74.6–76.9	8.7–9.5	11.9–12.3	—	—

<sup>1</sup> Unpublished analyses of eggs made in the Bureau of Chemistry, U. S. Department of Agriculture.

Dried yolk contains 1.65% lecithin-phosphoric acid, the dried whole egg 1.25%.

According to Beach, Needs, and Russell (1921) dried eggs (3 samples) contain 4.8% to 9.0% water, 3.17% to 3.71% ash, 39.1% to 50.6% fat, 43.5% to 46.5% protein, and 1.2% to 1.3% organic phosphoric acid compounds.

The average composition of 40 samples of dry whole egg powder, as given by Sudendorf and Penndorf (1924), is as follows:

TABLE XVII

	Minimum	Maximum	Average	Average on dry basis
	%	%	%	%
Water	4.54	8.63	6.32	—
Ash	3.23	5.78	4.02	4.29
Protein	29.30	45.10	40.90	43.66
Fat	34.20	52.50	41.61	44.42

### Dried Eggs (Characteristics and Uses)

Dried eggs are used very little if at all by the housewife or in restaurants; they are used to some extent by hospitals, eleemosynary institutions, hotels, and military establishments. Such products are peculiarly adapted to large-scale usage as, for example by manufacturers of noodles and prepared flours.

Dried eggs are a most convenient form of eggs, needing only to be measured or weighed; they are quite uniform in composition and characteristics and under proper conditions of storage keep perfectly with little change; they are more uniform in quality than are shell eggs. When mixed in water they are used just as are shell eggs. All three forms of dried eggs, the whole, yolks, and whites, are important articles of commerce. Most manufacturers prefer frozen eggs to the dried, but if the latter are cheaper they may, to a certain extent, replace the frozen product. For certain uses, dried, frozen, and shell egg products can be used interchangeably, but for the manufacture of many food products the frozen eggs are gradually displacing the dried. In certain industries, however, the dried egg is the most suitable form of egg to use. The U. S. Tariff Commission (1931) shows that about 80% of the dried eggs were being used in industries in which neither frozen nor shell eggs could be used, *viz.*, for the preparation of pancake, cake, pastry and doughnut flours, and for prepared ice cream powders and "mixes." Small bakers who cannot utilize a can of frozen eggs (22 to 44 pounds) in one day, and who lack refrigerating facilities, find it more convenient and economical to use dried eggs than shell eggs. For the manufacture of mayonnaise, ice cream, and noodles, the *dried* egg product may be used satisfactorily. Dried and frozen eggs are competitive only to a limited extent, although (U. S. Tariff Com., 1929) competition is more or less keen between the dried and frozen product in such industries as noodle, ice cream, and mayonnaise.

Dried eggs are mostly used in combination with other food materials, and not as a food direct. Dried egg yolks and dried whole eggs can be, and are being, used economically in making pastry and doughnuts, as well as noodles.

Experiments in making gold cake with dried yolk showed very clearly how ill-adapted this product is for cake making. The cake made with dried egg yolk was smaller, and had a less attractive appearance and poorer texture and grain than that made from fresh yolk. The grain was very irregular, large holes predominating, owing to the difficulty of properly reconstituting the dried yolk into a smooth, uniform mass free from lumps.

Because of the difficulty of reconstituting the dried whole egg into a lump-free liquid it is likewise better to use fresh or frozen eggs for all kinds of cake (Bailey and LeClerc, 1935).

Fairly good cakes (angel food) can be made with dried albumen, but angel food of better quality results from the use of frozen whites.

Dried albumen finds use in prepared whipping powders and meringues, and by confectioners in making cream centers, nougatines, marshmallow whips, etc. A few baking powder manufacturers have for years been adding small quantities of dried egg white to their product.

Dried egg whites are being largely displaced by the frozen whites in the manufacture of candies, especially packaged goods. Pie bakers who formerly used the dried whites for making meringues for soft pies are turning to the frozen product. For cake making, the dried whites have been almost entirely displaced by the frozen and fresh whites. There is very little competition between domestic frozen or shell eggs and the imported dried eggs (U. S. Tariff Com., 1931).

To determine the practical value of dried white, a test of the whipping properties is generally used. One part of white is mixed with 7 to 10 parts of water and, after standing for three hours, the mixture is beaten in a specified type of mixer. Judgment of the value of the dried white is based on the behavior of the beaten material or meringue (Swenson, 1938). Details of the tests follow:

Unfermented albumen: 1 oz. dried albumen to 7 oz. water. Stand 3 hours.

Whip test: (Hobart mixer, 10-quart bowl).

2 minutes, second speed

4 minutes, high speed

Fermented albumen:  $1\frac{1}{2}$  ozs. dried albumen to 15 ozs. water. Stand 3 hours.

Whip Test: (Hobart mixer, 10-quart bowl).

$1\frac{1}{2}$  minutes, medium speed

$1\frac{1}{2}$  minutes, high speed

The meringue is judged as follows:

Level the beaten meringue. Measure depth with either gauge or rule. Ordinarily a whip of 6 to 9 inches is obtained. Six and one-half inches or above is considered "fancy"; below six inches, a poorer grade.

A handful of the meringue is broken and examined for texture. A good meringue gives a clean break and the structure is firm. If a crackle is heard the body will not hold up.

A drip test may also be run as a measure of the strength of the meringue body. A weighed amount of foam is placed in a funnel and the time required for the first drop of liquid to come through is recorded. A high-grade product has little or no drip.

The Federal standards for eggs and egg products are given in the U. S. Department of Agriculture Service and Regulatory Announcements (1936).

1. Liquid Eggs, Mixed Eggs. The product obtained by separating the edible portion of eggs from the shells. It is an intimate mixture of the whites and yolks in their natural proportions.

2. Frozen Eggs. The solidified product obtained by quickly and completely freezing liquid eggs.

3. Dried Eggs. The product obtained by evaporating the water from liquid eggs. It contains not more than 7% of moisture.

4. Egg Yolk. The product obtained by removing the whites from the yolks in the commercial process of egg-breaking. It contains not more than 12% by weight of adhering white.

5. Frozen Egg Yolk. The solidified product obtained by quickly and completely freezing egg yolk.

6. Dried Egg Yolk. The product obtained by evaporating the water from egg yolk. It contains not more than 5% of moisture.

The definition for noodles is given on page 8 of the above-mentioned reference, as follows:

2. Noodles, Egg Noodles. The shaped and dried doughs prepared from wheat flour and eggs, with or without water and with or without salt. The egg ingredient may be whole egg and/ or egg yolk. In the finished product the moisture content does not exceed 13% and the egg-solids content upon the moisture-free basis is not less than 5.5% Noodles are commonly ribbon-shaped.

Based upon the assumption that the egg material is of normal composition, noodles will generally contain the proper amount of egg solids, if the following amounts of eggs or yolk are used with every sack (98 pounds) of flour: Commercial liquid whole egg, 19 pounds 8.4 ounces; commercial liquid yolk, 13 pounds 11.5 ounces; commercial dried whole egg, 5 pounds 7.4 ounces; commercial dried yolk, 5 pounds



5.5 ounces. With a sack of 140 pounds of flour the following amounts of eggs or egg products should be used: Commercial liquid whole egg, 27 pounds 14.2 ounces; commercial liquid yolk, 17 pounds 9.6 ounces; commercial dried whole egg, 7 pounds 12.8 ounces; commercial dried egg yolk, 7 pounds 10.1 ounces. (LeClerc, 1933.)

According to the Macaroni Journal (Anon., 1929) the manufacturer should use with each 95 pounds of flour either 5 pounds of dried egg yolk or 20 pounds of fresh whole egg, or 12½ pounds of fresh yolk.

The method for the detection of whole egg or of egg yolk, and for the estimation of the quantity of these egg products used in the manufacture of noodles, is described fully by Buchanan (1924), and in Cereal Laboratory Methods (Anon., 1935).

### Miscellaneous Information

One hundred pounds of shell eggs yield 10 to 12 pounds of shell, 58 to 60 pounds of albumen, and 30 to 32 pounds of yolk.

As the weight of eggs varies, so does the percentage of the egg components. The following is representative of eggs of different size:

TABLE XVIII  
EFFECT OF VARIATION IN SIZE ON COMPOSITION

Eggs per lb.	Av. wt. per egg	Approximate wt. of			Percentage of		
		Yolk	Whites	Shell	Yolk	Whites	Shell
No.	g.	g.	g.	g.	%	%	%
7	64.8	18.1	40.3	6.4	27.9	62.2	9.9
8	56.7	16.6	34.4	5.7	29.3	60.7	10.0
9	50.4	15.6	29.7	5.1	30.9	58.9	10.2
10	45.4	14.9	25.8	4.7	32.8	56.8	10.4
11	41.2	14.5	22.4	4.3	35.1	54.4	10.5

Depending upon the weight per egg, 100 eggs will yield the following quantities of yolk, whites, liquid whole egg, and shell and of the dried products:

TABLE XIX  
COMPOSITIONS OF EGGS PER 100

Eggs per lb.	Yolk	Whites	Whole egg	Shell	Dried yolk	Dried white	Dried whole egg
No.	Lbs.	Lbs.	Lbs.	Lbs.	Lbs.	Lbs.	Lbs.
7	3.99	8.88	12.87	1.41	1.88	1.25	3.77
8	3.66	7.58	11.24	1.25	1.72	1.07	3.29
9	3.44	6.55	9.99	1.12	1.62	0.92	2.93
10	3.28	5.69	8.97	1.04	1.55	0.80	2.63
11	3.19	4.94	8.13	0.95	1.50	0.69	2.38

The data given above are calculated on the assumption that one pound of dried egg product is produced from 3.41 pounds of liquid whole egg, 7.12 pounds liquid whites, or 2.12 pounds liquid yolk. To calculate the quantities of egg products—frozen or dried—per crate of 30 dozen, multiply the values given above by 3.6.

A case of 30 dozen domestic eggs weighs about 42 to 43 pounds net and yields approximately 35 pounds of liquid whole eggs. One hundred pounds of shell eggs produce commercially approximately 84 pounds of liquid whole eggs. In China 100 pounds of shell eggs produce only 81 pounds of liquid whole eggs (U. S. Tariff Com., 1929).

Twenty whole eggs, or 36 whites, or 48 yolks, make one quart. On the average, the liquid egg from 9 to 11 shell eggs weighs one pound; 17 to 20 whites, or 19 to 22 yolks, weigh one pound.

Thirty shell eggs produce one kilogram (2.2 pounds) of frozen eggs.

One pound of dried eggs can be made from 39 eggs, one pound of dried yolk from 48 yolks, and one pound of dried albumen from 70 whites.

Approximately  $3\frac{1}{3}$  pounds of whole liquid eggs yield 1 pound of dry whole egg; 7 pounds of whites yield 1 pound of dry albumen; and 2 pounds of yolks yield 1 pound of dry yolk.

One pint or one pound of liquid or frozen egg is obtained from 9 to 11 eggs.

#### Literature Cited

##### Anonymous

- 1929 Eggs and egg products. U. S. Tariff Com. Rept., 36 pages.
  - 1929 Ratio of eggs to flour. Macaroni J. 10 (12): 36.
  - 1931 Dried egg-products. U. S. Tariff Com. Rept. 25, 2nd ser. 17 pages.
  - 1932 The preparation of frozen and dried eggs. Food Manuf. 7(5): 150.
  - 1935 Cereal laboratory methods of the A. A. C. C., 3rd ed.
  - 1936 Service and regulatory announcements. Food and Drug 2 (5th rev.), Food and Drug Adm., U. S. Dept. Agr.
  - 1938 Frozen eggs directory. Food Ind. 10 (4): 249.
- Armour and Co.
- 1929 Egg whites and baking. Research Bul. 1, 4 pages.
- Asmundson, V. S.
- 1931 The formation of the hen's egg. Sci. Agr. 11: 590-606.
- Bailey, L. H., and LeClerc, J. A.
- 1935 Cake and cake making ingredients. Cereal Chem. 12: 175-212.
- Beach, F. F., Needs, F. E., and Russell, Edward
- 1921 The composition of egg powder. The Analyst 46: 279-283.
- Bidault, C.
- 1928 Oeuf frais et oeuf réfrigéré. Rev. Gén. Froid et Indus. Frigor. 9: 84-87.
- Bordas, F.
- 1922 Le commerce des oeufs congelés. Rev. Hyg. 44: 613-632.
- Buchanan, Ruth
- 1924 Analyses of noodles of known composition and estimation of their egg solids content. J. Assoc. Off. Agr. Chem. 7: 407-424.
- Epstein, A. K.
- 1931 Eggs, their properties and their function in cake making. Am. Soc. Bakery Eng. Bul. 62, 4 pages.
- , Reynolds, M. C., and Harris, B. R.
- 1937 Mayonnaise and other salad dressings, 68 pages.

- Graham, W. R.  
1908 Experiments in hatching and rearing chickens. Ontario Dept. Agr. Bul. 163, Ont. Agr. Coll., 66 pages.
- Heitz, Thomas W.  
1929 The cold storage of eggs and poultry. U. S. Dept. Agr. Circ. 73, 54 pages.
- Hunt, Leila Wall, and St. John, J. L.  
1931 Angel food cake from the thick and thin portions of egg white. J. Home Econ. 23: 1151-1156.
- König, J.  
1904. Chemie der menschlichen Nahrungs- und Genussmittel. 4th ed. 2: (Herstellung, Zusammensetzung u. Beschaffenheit) 574.
- Langworthy, C. F.  
1901 Eggs and their uses as food. U. S. Dept. Agr. Farmer's Bul. 128, 32 pages.
- LeClerc, J. A.  
1933 Macaroni products. Cereal Chem. 10: 383-420.
- McMillen, D. W.  
1930 Increased egg consumption. Address of Pres. Am. Feed Manuf. Assoc., Miller's Rev. and Feed Forum 94 (6): 15-17.
- Monvoisin, A.  
1928 Les oeufs réfrigérés. Rev. Gén. Froid et Indus. Frigor. 9: 84.
- Nemetz, Frank J.  
1929 (Jan.) Egg whites. Am. Baker's Assoc. Bul. CR-1, 4 pages.  
1929 (Feb.) Egg yolks. Am. Baker's Assoc. Bul. CR-2, 4 pages.
- Ovson, Leo D.  
1938 Better frozen eggs for the institutional and manufacturing industries. Quick Frozen Foods 1 (3): 15-18, 40.
- Radabaugh, J. H.  
1938 Production of frozen eggs in the U. S. in 1937. Poultry Sect., A. A. A., U. S. Dept. Agr.
- Redfield, H. W.  
1920 Examination of frozen egg products and interpretation of results. U. S. Dept. Agr. Bul. 846, 96 pages.
- Romanoff, Alexis L.  
1929 The dry matter in different layers of egg albumin. Sci. 70: 314.
- Sharp, Paul F.  
1937 Preservation and storage of hens' eggs. Food Research 2: 477-498.
- Sørensen, Margrethe  
1934 Über den Kohlenhydratgehalt der Proteine des Hühnereiweisses. Biochem. Z. 269: 271-284.
- Sudendorf, Th., and Penndorf, O.  
1924 Über Trockenvollei des Handels und seine Beurteilung nach neueren Gesichtspunkten. Z. Nahr. Genussm. 47: 40-50.
- Swenson, T. L.  
1938 Information on drying eggs. Mim. Circ. 58, Bur. Chem. and Soils, U. S. Dept. Agr., 4 pages.
- Swift and Co.  
—— Frozen eggs, 48 pages.
- Tranin, Samuel  
1938 Dried egg product and method of producing the same. U. S. Pat. 2,127,945, Aug. 23.
- Winckel, Max  
1925 Trockenindustrie (Ei-Industrie) III. Chem. Ztg. 49: 229-230.

# EXTENSOGRAMS AS A BASIS OF PREDICTING BAKING QUALITY AND REACTION TO OXIDIZING AGENTS

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(Received for publication February 13, 1939)

There are two schools of thought in accounting for the effects of oxidizing agents upon the physical properties of flour doughs, one of which maintains that such agents act indirectly by inhibiting or restraining proteolysis, while the other contends that they function in terms of their direct effect upon colloidal properties of the dough. Farinograms have not proved to be singularly useful in elucidating the manner or degree of the changes effected by such reagents, either when the observations are made upon freshly mixed dough, or upon doughs previously fermented for varying periods of time. These limitations may be the consequence of the fact that the farinograph so operates as to disclose properties of a dough in a state of continuous agitation, rather than the properties of a resting dough. One can discern by the sense of touch that oxidizing agents alter certain properties of doughs, but when these doughs are placed in a mixing machine and subjected to continuous mixing, the differences are not evidenced by the resulting farinograms. With the extensograph opportunity is afforded to test doughs which have previously been in a state of rest for more or less extended periods, and accordingly this instrument was applied to a study of the effect of chemical treatments upon dough properties.

In the first series of studies, doughs prepared from a blend of European flours were subjected to three molding treatments: (1) immediately after mixing, (2) two hours, and (3) four hours after mixing. Portions of each dough were then tested at varying intervals of time after molding, the  $F/E$  ratio<sup>1</sup> of the extensograms, and the area under the extensogram curves being measured with the results shown in Figure 1. A like series of doughs was prepared to which 0.003%  $KBrO_3$  was added, and similar analyses of the extensograms are recorded in Figure 2. No differences were registered immediately after mixing, but when the doughs were allowed to rest 4 hours before molding and tested 30 minutes later, the  $F/E$  ratio was substantially increased by the presence of bromate.

When potassium persulfate was used instead of bromate, a more immediate effect was registered in terms of the extensograms. Thus some effect of the persulfate is demonstrated shortly after mixing, as may be discerned by contrasting the extensograph tests of the doughs

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<sup>1</sup>  $F$  = force applied in extending doughs;  $E$  = extension.

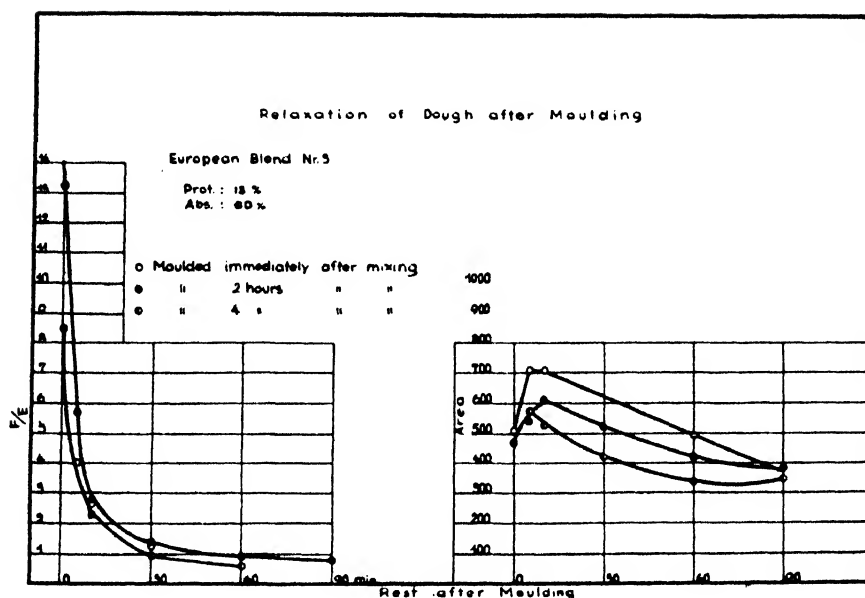


Fig. 1. Changes in  $F/E$  relationships of extensograms of doughs prepared from a blend of European flours plotted against the rest period after molding; area under the same extensograms plotted against rest period after molding. Three series of doughs which were (1) molded immediately after mixing, (2) molded after two hours, and (3) molded four hours after mixing.

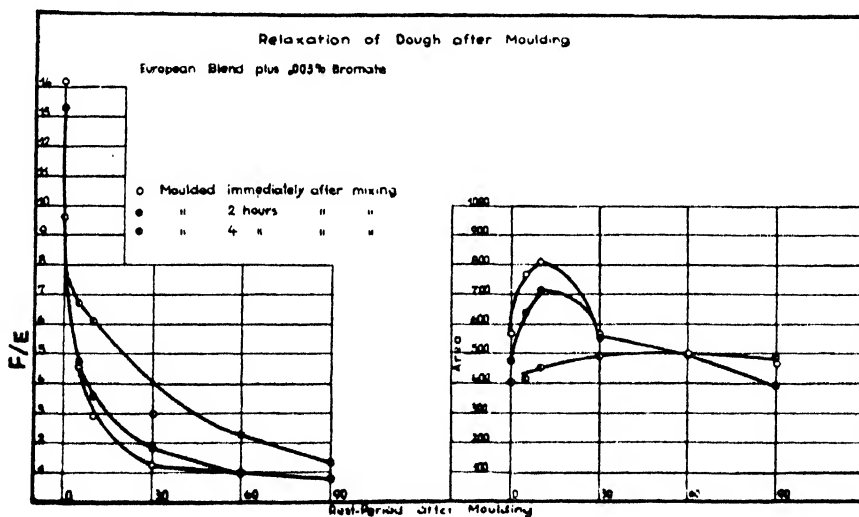


Fig. 2. Data as in Figure 1, of doughs from the same flour blend plus 0.003% bromate.

prepared with persulfate and recorded in Figure 3 with the data of the controls in Figure 1.

A scale for expressing the sensitivity of flours to oxidizing agents would be of great convenience and value in flour technology. In devising such a scale, consideration must be given to the baking procedure, with particular reference to formula, mixing time, fermentation time,

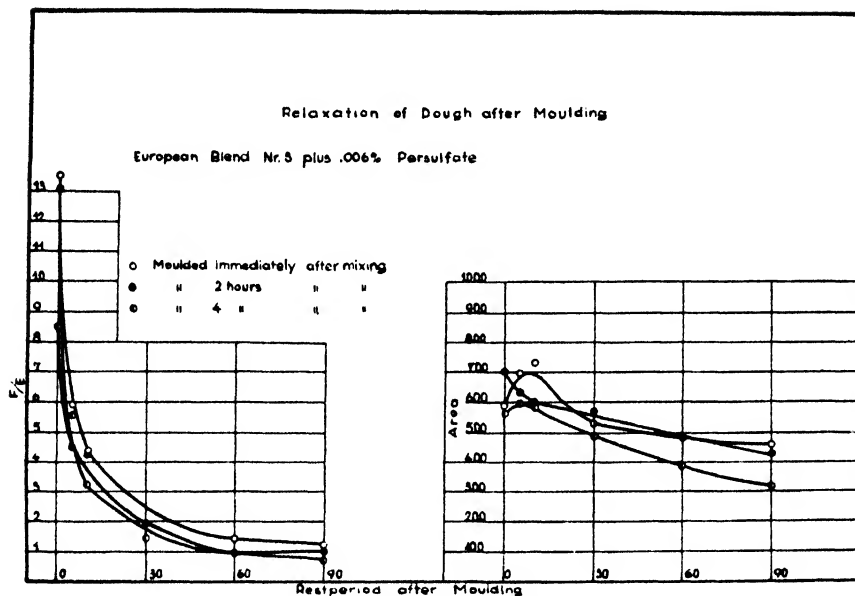


Fig. 3. Data as in Figure 1 of doughs from same flour blend plus 0.006% persulfate.

and whether sponge doughs or straight doughs are used. The significance of fermentation time in this connection is graphically demonstrated by the photographs of loaves illustrated in Figure 4. Thus a

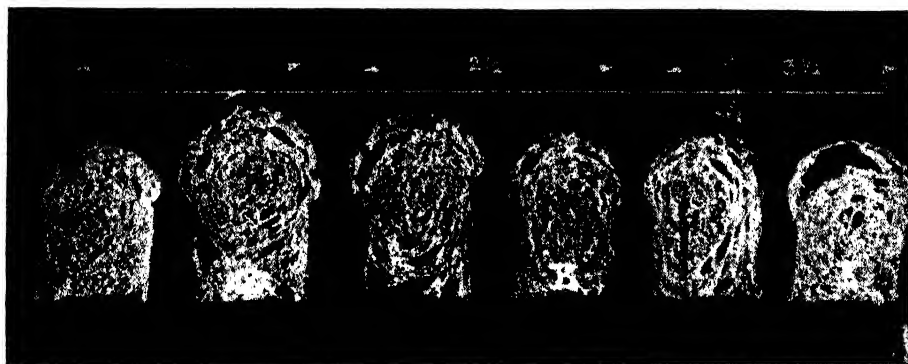


Fig. 4. Photographs of loaves baked from doughs previously fermented for  $1\frac{1}{2}$ ,  $2\frac{1}{2}$ , and  $3\frac{1}{2}$  hours respectively and prepared with bromate (marked B in illustration) and without bromate (odd-numbered loaves).

decided positive response, as registered in loaf volume, resulted from the inclusion of bromate when the doughs were fermented  $1\frac{1}{2}$  hours, while after  $2\frac{1}{2}$  hours of fermentation the response was negative. The scale to be employed for any particular bake-shop program may be evolved by identifying the flour type which gives no response to bromate and then rating other flours upward or downward, *i.e.* "positively" or "negatively" to the extent that they deviate from the neutral type.

Extensograms have proved to be useful criteria of bromate reaction when subjected to a simple mathematical treatment. The ratio  $F/E$  alone is not sufficient. Thus the same ratio would result from calculations based upon the two curves shown in Figure 5, whereas the flour

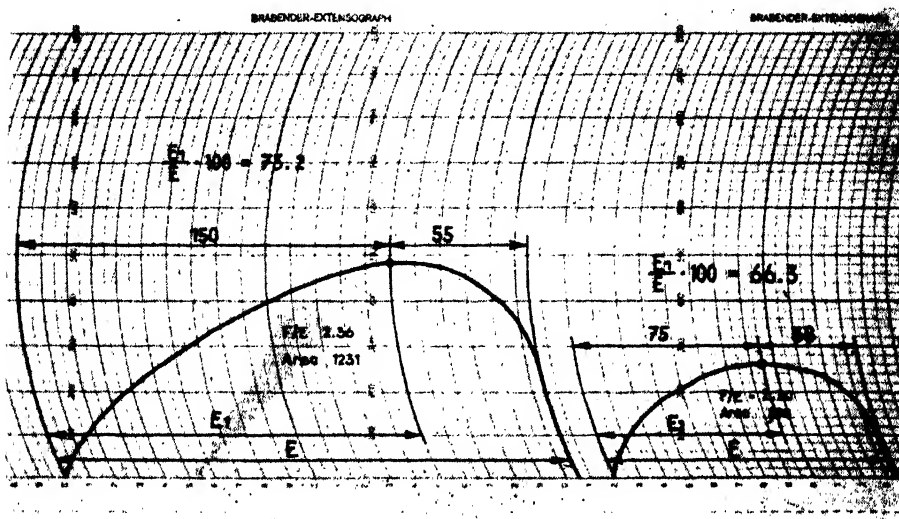


Fig. 5. Schematic extensograms showing tests of doughs giving about the same  $F/E$  ratios but with widely different curve areas. Flours represented by the type of curve at left react positively to bromate; those represented by curve at right react strongly negatively.

represented by the larger curve at the left reacted positively to bromate, while the flour which gave the extensogram at the right reacted decidedly negatively. If the ratio  $F/E$  is multiplied by 10, and this divided into the curve area as in the formula

$$\text{Oxynumber} = \frac{\text{Area}}{F/E \times 10}$$

the values which result will range from approximately 0 upwards to  $70 \pm$ . In general a value of  $30-40$  will result from testing a flour that gives a neutral response under the majority of shop conditions. Accordingly, the range of such values can be classified in general terms as follows:

- 0-20 very negative
- 20-30 moderately negative
- 30-40 neutral
- 40-50 moderately positive
- >50 very positive

Referring back to Figure 5, the curve at the left yields an oxynumber of 52, while that at the right yields an oxynumber of 17, which serves to classify them properly on the basis of their baking behavior.

These mathematical treatments of the extensograms may be amplified by plotting the  $F/E$  ratios of a series of doughs which are allowed to rest for varying time intervals, and interpolating for the ratio at  $2\frac{1}{2}$  hours. This value will be referred to as *specific shape*. *Specific fermentation time* is the computed interval in hours of fermentation at which the  $F/E$  ratio passes through 3.

The deportment of various flours when subjected to bromate treatment is evident from the data recorded in Figures 6, 7, and 8. Two

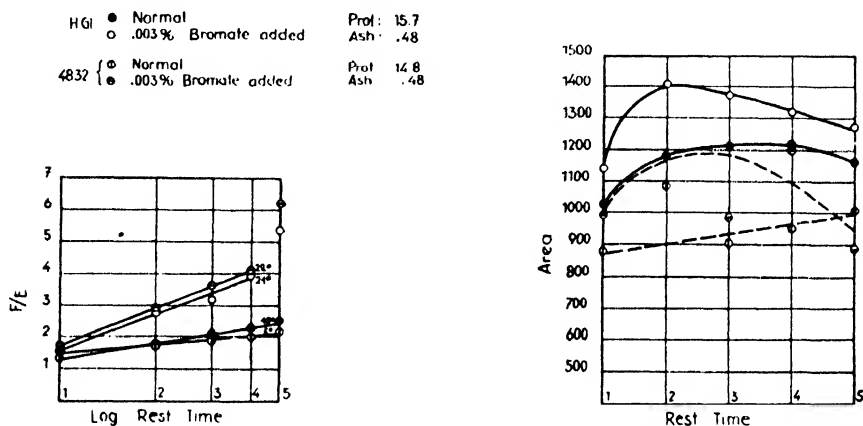


Fig. 6. Changes in  $F/E$  relationships of extensograms, plotted against the logarithm of rest time, of doughs made from two spring wheat flours yielding "pliable" doughs and prepared with and without bromate; also areas under the extensograms of the same doughs.

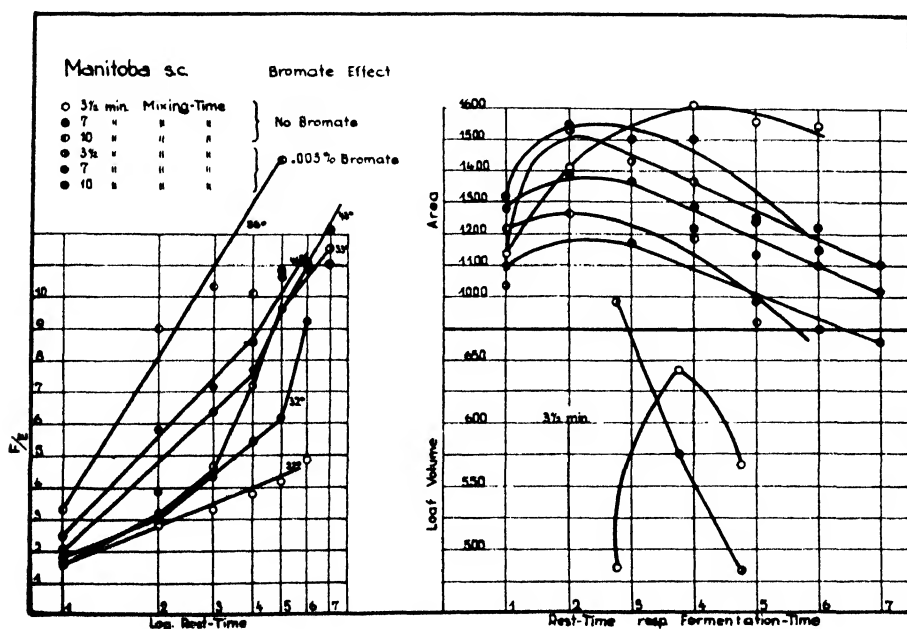


Fig. 7. Changes in  $F/E$  relationships of extensograms, plotted against the logarithm of rest time of doughs made from a Manitoba flour with an oxynumber of 45, with mixing times of  $3\frac{1}{2}$ , 7, and 10 minutes, and prepared with and without bromate; also the area under the same extensograms, and (lower right of figure) the loaf volumes of test loaves baked from doughs mixed  $3\frac{1}{2}$  minutes and prepared with and without bromate.



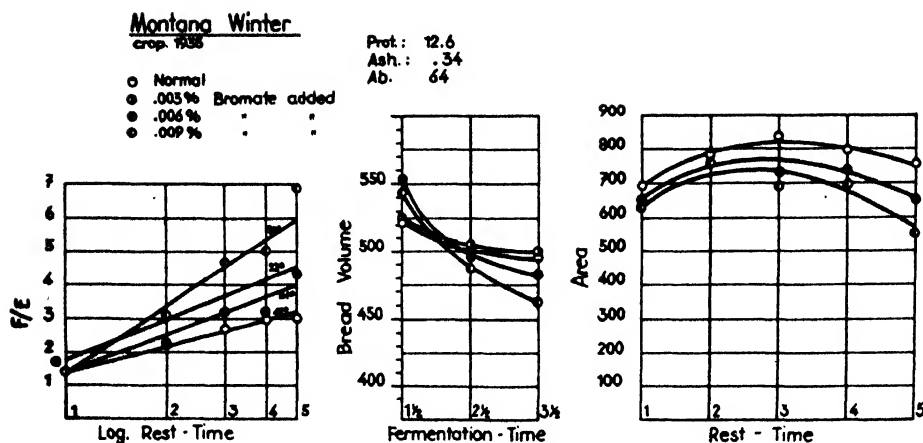


Fig. 8. Changes in  $F/E$  relationships of extensograms, plotted against the logarithm of rest time, of doughs made from a Montana winter wheat flour with an oxynumber of 34, the doughs being made with 0%, 0.003%, 0.006%, and 0.009% of bromate respectively; also the area under the same extensograms, and (center of figure) the volume of test loaves baked from an equivalent series of doughs fermented for varying time intervals as shown.

spring wheat flours yielding "pliable" doughs are represented in Figure 6. The high protein flour (*H.G. 1*) had an oxynumber of 66, which implies a very large bromate response, and the area of the extensogram was actually increased by 200 units (at two hours' rest time) upon the addition of 0.003% of bromate, thus confirming the original estimate. Flour 4832 had an oxynumber of 54 and it likewise responded in terms of an increased extensogram area upon the addition of bromate. From these and other observations it appears safe to predict that a parallel increase in loaf volume would result when bromated doughs are baked into bread.

In the instance of a Manitoba flour, the effect of bromate is shown in Figure 7. The oxynumber was 45, implying a moderate bromate response, which was confirmed by the extensograms. Baking tests supplied further confirmation, an increase of nearly 200 cc. resulting from the addition of bromate to doughs fermented for two hours. When the fermentation was extended to three hours the loaf volume decreased and was 70 cc. less than the non-bromated control.

Not all pliable doughs respond to bromating, but when the effect is negative the flour is abnormal in some manner, and the large extensibility ( $E$ ) cannot be attributed to an extensible gluten, but rather to the contribution made by other substances which facilitate the flow of gluten micelles or aggregates past each other without coalescing. Thus in the instance of a Montana winter wheat flour the extensogram constants recorded in Figure 8 afford such an example. Its oxynumber was 34, which commonly denotes a neutral bromate response, and the baking data recorded as loaf volumes in Figure 8 show such a reaction.

A very small positive response was registered upon the inclusion of bromate in dough fermented for a short time, and when the fermentation was extended the response was negative. Area of the extensograms was affected very little by the inclusion of bromate, and any effect observed was negative. The  $F/E$  ratio was affected most definitely, the specific form (as defined above) being changed from 2.4 to 4.0 by adding 0.009% of bromate.

Flours having a very high water absorption may comport themselves differently than would be anticipated from their oxynumbers alone, and these may be recognized from the sticky characteristics of the doughs. Such a flour was encountered in the instance of the sample which was converted into the test loaves shown in Figure 9.



Fig. 9. Photographs of test loaves baked from doughs fermented 1½ and 2½ hours as indicated and with 73% or 66% of water added, as shown below the loaves. Loaves Nos. 2 and 5 marked B were made with added bromate.

Using the farinograph to determine water absorption, 73% of water was required to produce a dough of standard consistency. The bromated loaf from the dough fermented for 2½ hours had a much smaller loaf volume than the control. When the water used was reduced by 7%, namely to 66%, a normal loaf resulted, as shown by the section of the loaf at the extreme right of the picture. It may be that the inclusion of less water resulted in a reduced hydration of those substances which effect abnormalities in the dough, and thus left the gluten free to register its normal response to the bromate that was added.

#### Action of Nitrogen Trichloride and Chlorine

Extensive data have not been accumulated as yet involving the action of nitrogen trichloride ("Agene") upon dough properties, but the observations to date indicate that it effects changes similar to those produced by bromate. Chlorine treatments are applied most commonly to cake flours, and occasionally to the more heavily buffered flour streams and to the clear grades. In treating cake flour it is

generally conceded that the major effect is induced by reducing the pH to about 5.4, and that further additions of  $\text{Cl}_2$  produce much smaller effects. The farinograph has not proved singularly useful in recording such effects, however, and accordingly in the past it was necessary to resort to practical cake-baking tests. That the extensograph records progressive changes in dough properties effected by increments of  $\text{Cl}_2$  is shown by the data in Figure 10. In the instance of these tests three increments of  $\text{Cl}_2$  were added which were sufficient to reduce the pH to 5.4, 5.2, and 4.9 respectively. At pH 5.4 to 4.9 the  $F/E$  ratio was increased substantially over that of the control. When that ratio was plotted against the logarithm of the time of rest of the dough as abscissas the resulting graph deviated from the horizontal by  $22^\circ$  in the instance of the treated flours, and only  $9^\circ$  in the untreated control

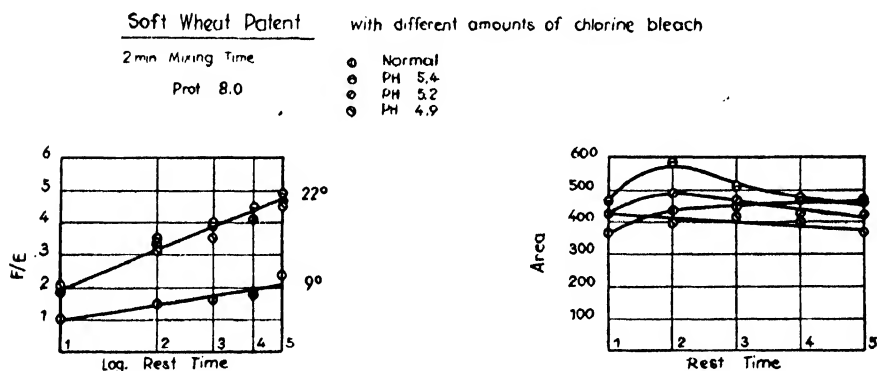


Fig. 10. Changes in  $F/E$  relationships of extensograms, plotted against the logarithm of rest time, of doughs made from a soft wheat patent flour treated with varying amounts of  $\text{Cl}_2$  to reduce the pH to the levels indicated; also the area under the same extensograms.

doughs. Likewise the area under the extensograms was greater in the flour treated to pH 5.4, but the area decreased somewhat upon the addition of more chlorine to pH 4.9.

### Effect of Papain, Cysteine, and Bromate

The effect of the addition of 0.005% papain upon a Canadian hard spring wheat flour is shown in the set of extensograms at the left in Figure 11. The specific form of the untreated or control dough (without papain) was 4.8, and this was changed to 3.0 by the inclusion of the papain, although the curve area was not substantially altered. This implies an increased extensibility in consequence of the effect of the papain.

When 0.002% of potassium bromate was superimposed upon the papain, the resulting extensogram was essentially identical with that of the control or papain-free dough. This observation affords addi-

tional support from another, and physical approach, to the conclusion previously reached by Jørgensen (1935) and others that bromates do restrain or control the action of papain and similar proteolytic enzymes, especially when the level of activity of the latter is not too high.

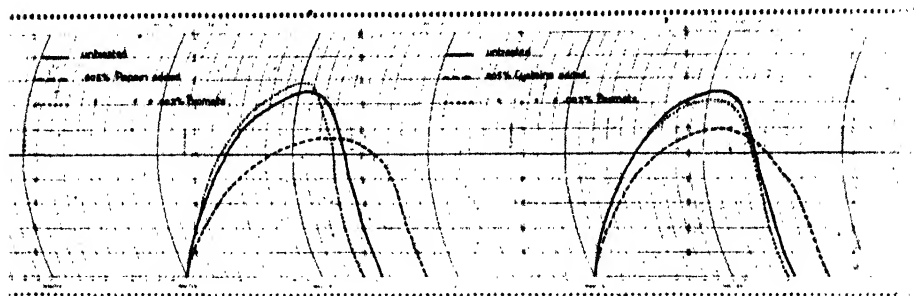


Fig. 11. Extensograms of a dough made with a Canadian hard spring wheat flour. At the left is the control or untreated flour (solid lines), the same plus 0.005% papain (broken line), and also (dotted line) a combination of papain plus 0.002% bromate. At the right is an equivalent series involving cysteine instead of papain.

Likewise the addition of 0.005% of cysteine to a dough effected a modification of the extensogram analogous to that produced by papain, and its effect, in turn, was restrained by the superimposition of bromate. When the dough was allowed an extended rest period the action of the cysteine became more evident, however, as shown by the analysis of the extensograms recorded in Figure 12. In terms of both  $F/E$  ratio and area, the alteration in dough properties departs from the control with the lapse of time and when the latter is con-

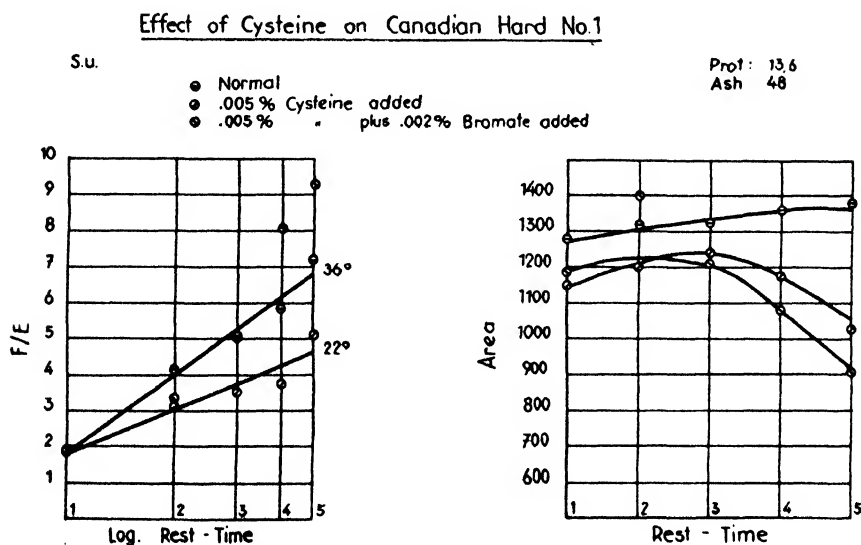


Fig. 12. Changes in  $F/E$  relationships of extensograms, plotted against the logarithm of rest time, of doughs prepared from a Canadian hard spring wheat flour without treatment, with 0.005% cysteine, and with cysteine plus 0.002% bromate; also area under the same extensograms.

siderable there is evidence that the bromate did not restrain the effect of the cysteine.

### Heat Treatment of Flour

With certain types of medium and low-strength bread flours a carefully adjusted heat conditioning treatment effects an improvement analogous to that which results from the use of bromate. Such a treatment was accorded a flour milled from a Hungarian wheat ("Ungar"), and the data from a series of extensograms involved in these comparisons are recorded in Figure 13. Both the area (right

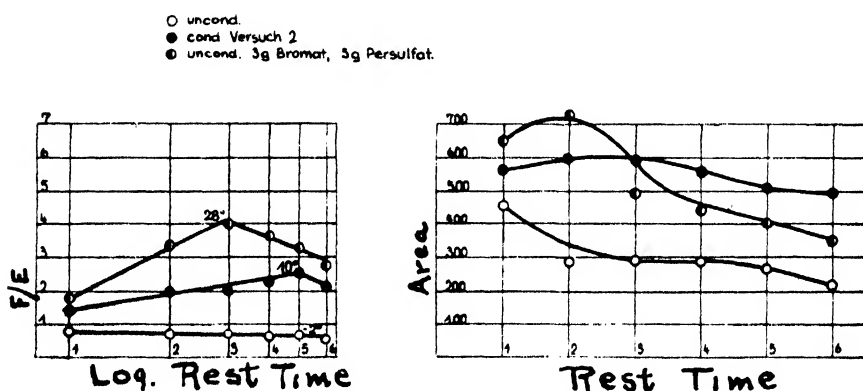


Fig. 13. Changes in  $F/E$  relationship of extensograms, plotted against the logarithm of rest time, of doughs prepared from an Hungarian wheat flour and subjected to heat conditioning (solid dots), and bromate or persulfate treatments (half shaded dots), in comparison with an untreated control (open circles); also the area under the same extensograms.

figure) and the  $F/E$  ratios (left figure) were increased by heat conditioning, as was true also of the bromate and persulfate treatments.

### Effect of Diastase on Dough Extensograms

The inclusion of malted wheat flour in doughs in normal proportions did not substantially alter the extensograms, which suggests that the gluten properties were not affected greatly by the enzymes thus contributed to the dough. This adds support to the earlier conclusion of Munz and Bailey (1936) that the modifications in dough plasticity resulting from the addition of wheat malt flour might be traced to the changes in the starch properties effected by the amylases rather than to proteolysis.

### General Relations of Farinograms and Extensograms to Baking Quality

During the period covered by the researches reported in this series of papers, a considerable number of flours of various types have been subjected to detailed study with the farinograph and the extensograph.

In many instances the baking behavior of these flours was known from practical tests made in this institute or elsewhere; in other cases the previous work done with like flours afforded a basis for relating our observations with the general department in the baking of the flour types represented. It appears desirable, therefore, to call attention to certain general relations which can now be deduced from a comparison of the observations based upon baking behavior with the results of these physical tests. In doing so, it is recognized that precise mathematical correlations cannot be provided from the data at hand, and these must appear as a greater volume of data is accumulated. The purpose of the present study is to indicate apparent trends, with the thought that the principles suggested may be subjected to further critical study here and in other laboratories provided with adequate equipment.

Relations between farinograms, extensograms, and bread-baking qualities are indicated by the data recorded graphically in Figures 14

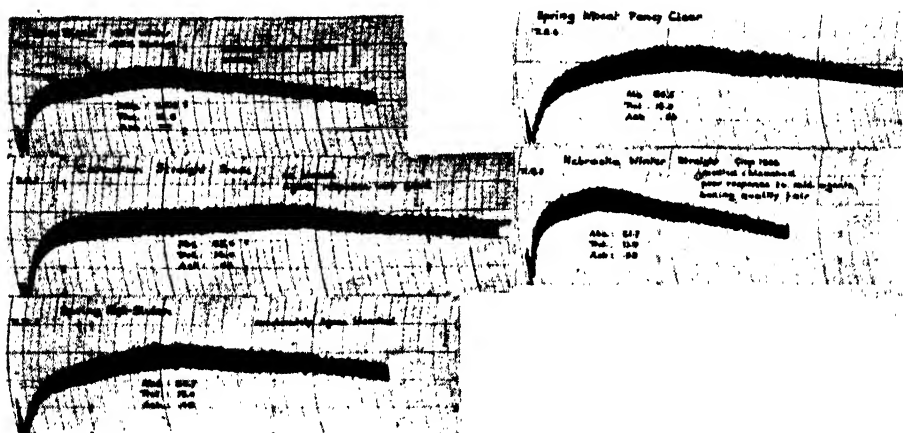


Fig. 14. Farinograms of the flours which (excluding the clear flour) are represented as their extensograms in Figure 15.

and 15 (disregarding the "Spring Wheat Fancy Clear" farinogram in Figure 14). The baking trade may be expected to rate the other four flours in the following order of decreasing bread-baking quality or "strength," viz., No. 3, No. 2, No. 1, and No. 5. This is also the order in which the area under the extensograms, recorded at the right in Figure 15, is arranged. It was reported that flour No. 2 gave best results only after treatment with such a reagent as Agene, and the characteristics of the extensogram with the computed oxynumber of 53 would suggest this conclusion independently of the actual tests with this reagent.

Flour No. 1 yields an extensogram area of 750 units at the optimum rest time, and its oxynumber of 32 indicates that it has already been

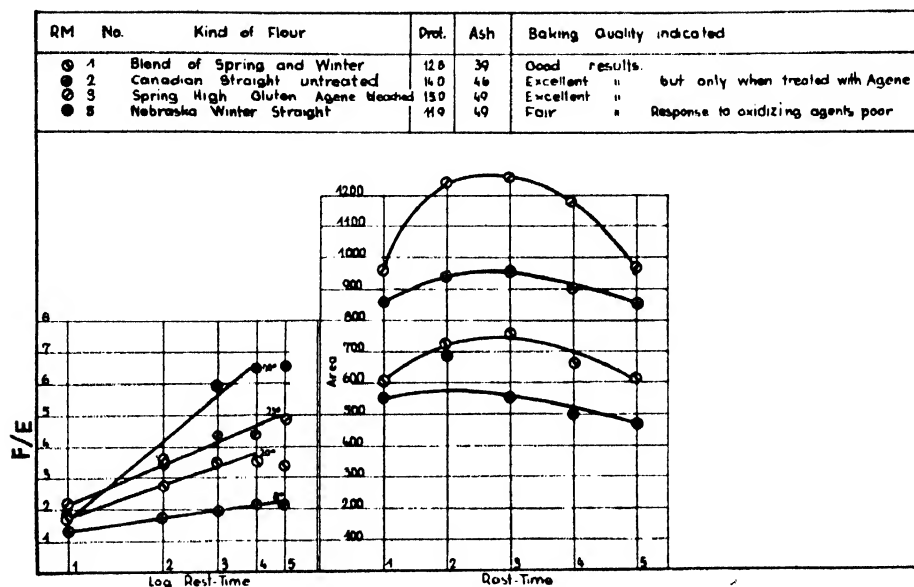


Fig. 15. Changes in  $F/E$  relationships of extensograms of four flours plotted against the logarithm of rest time; also areas under the same extensograms.

given a normal chemical treatment. Flour No. 5 was reported to give only fair results in baking, which would be expected from its extensogram area. Likewise its response to oxidizing agents was poor, which would be deduced from its oxynumber of 12.

The farinograms of another series of six flour samples are shown in Figure 16. Three of these were milled from northwestern (U. S.) spring wheats, and their protein contents ranged from 12.7% to 15.0%.

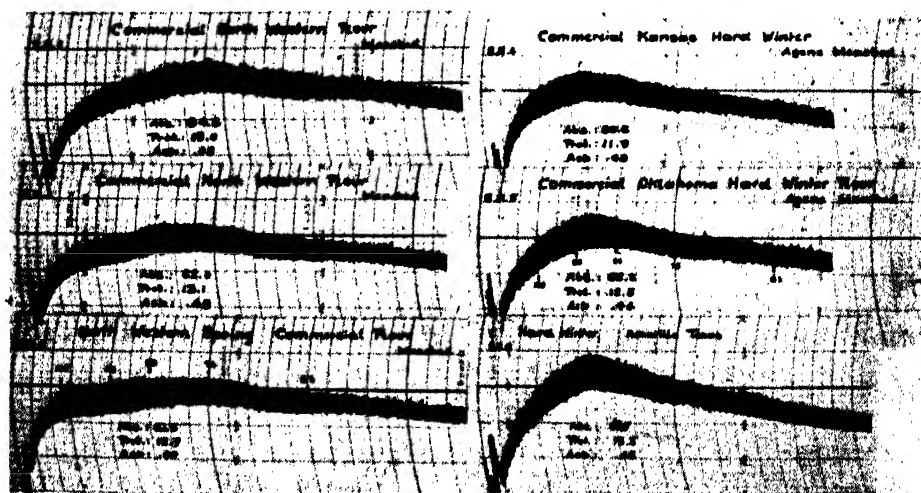


Fig. 16. Farinograms of the six flours which are represented as their extensograms in Figure 17.

The other three were milled from southwestern (U. S.) hard winter wheat, and their protein content ranged from 11.9% to 13.5%. These six flours all comported themselves in baking like flours of their respective types or classes, and protein content. Data from extensograms of these six flours are recorded graphically in Figure 17. The areas under

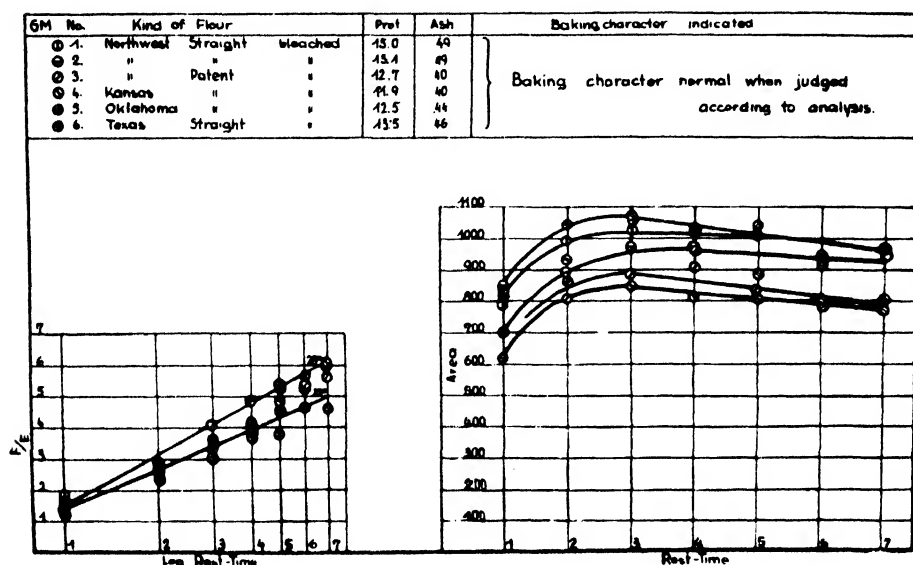


Fig. 17. Changes in  $F/E$  relationships of the extensograms of three northwestern and three southwestern wheat flours plotted against the logarithm of rest time; also the area under the same extensograms.

the extensograms tended to decrease regularly with protein content in each of the two groups as indicated in the following table.

N.W. spring wheat flours  
Crude protein, %

15.0  
13.1  
12.7

Area under extensogram  
at maximum

1020  
960  
880

S.W. winter wheat flour

13.5  
12.5  
11.9

1070  
960  
840

In these series it chanced that each unit of protein content was accompanied by a somewhat higher extensogram area in the winter wheats than in the spring wheats, which may be the consequence of an optimum Agene treatment of the former. While the extensograms alone would not serve to distinguish the Agene-treated winter wheat flours from the spring wheat flours, reference to the farinograms depicted in Figure 16 discloses differences in the curves.



Farinograms of another collection of flours are shown in Figure 18, and their extensogram analyses are recorded in Figure 19. Baking behavior of such flours can be predicted fairly well from these physical tests. Flour *II. G.*, containing 15.7% crude protein, gave an extenso-

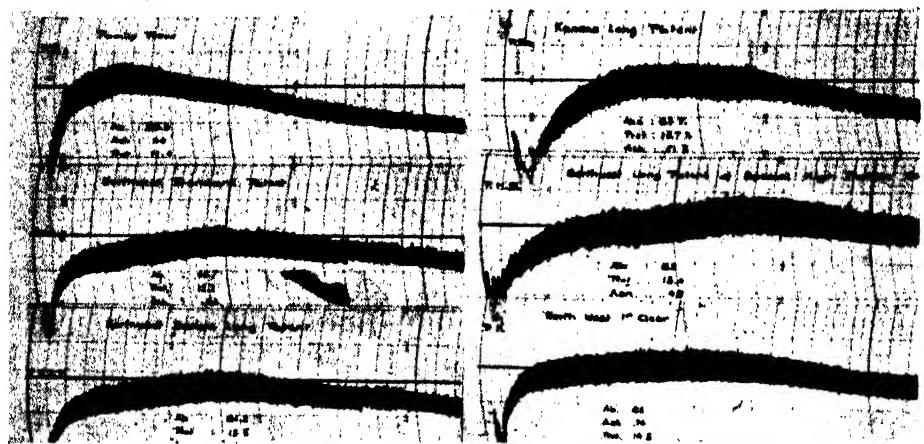


Fig. 18. Farinograms of the six flours which are represented as their extensograms in Figure 19.

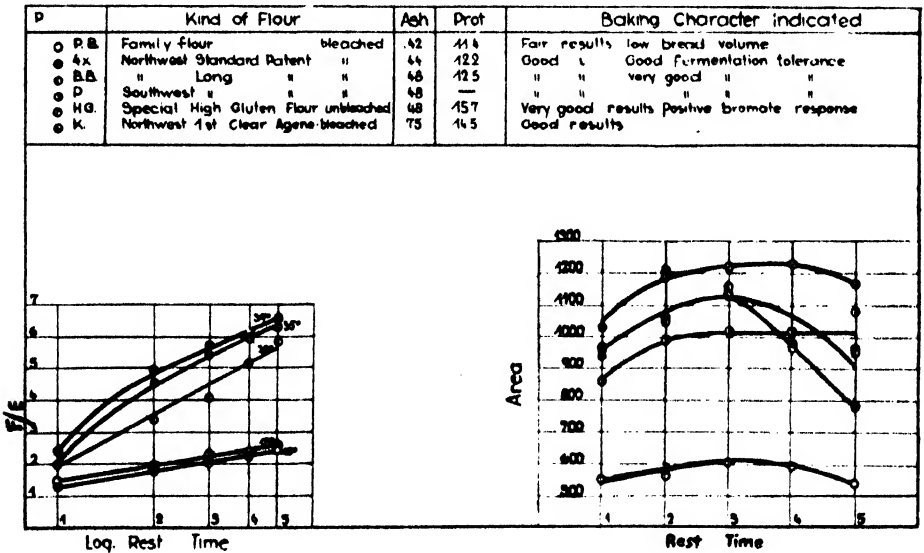


Fig. 19. Changes in  $F/E$  relationships of the extensograms of six flours plotted against the logarithm of rest time; also the area under the same extensograms.

gram with a maximum area of 1230, and an oxynumber of 67. This indicates superior baking behavior and a decidedly positive response to bromate, which was confirmed by baking experience. Moreover, these properties also indicate the adaptability of this flour to the

extended fermentation that is accorded it in commercial bakery practice.

Flours *4x* and *BB* were reported to give good results in bread baking, and this would be predicted from the large maximum area, 1130, under their extensograms. While the flour *4x* gave a low oxynumber, 21, this may be attributed to the fact that it had aged a long time before these tests were made.

Flour *P*, milled from southwestern wheat, yielded an extensogram having a maximum area of the order that would be anticipated.

The comparison afforded by the standard and the long-patent flours milled from northwestern wheat is significant, since long-patent exhibited the greater extensibility characteristic of such grades.

Flour *PB*, containing 11.4% crude protein and described in the trade as a general-purpose family flour, yielded an extensogram of low area, which should characterize flours of its low baking strength.

The first clear flour in this series marked *K* comported itself rather well as evident from the data of its extensogram. This might be attributed to its high gluten content combined with a strong but properly graduated Agene treatment.

In general the farinograms of this series of flours (Fig. 18) are useful in their support of the extensograms and their normal relationship to baking properties of such flours of rather widely diverse types.

### Relation of Protein Content to Area of Extensogram

Since protein content commonly determines the physical properties of dough which are reflected in the extensograms, it might be anticipated that the area under the extensogram would be correlated with the percentage of crude protein in the flour used in preparing the dough. Exceptions to this general relationship would be encountered in the instances of (a) glutens of poor "quality," *i.e.*, of abnormal properties, and (b) flours previously subjected to improper treatment with oxidizing agents. Conversely, when a flour does yield the predicted extensogram area for its level of protein content, it might be described as having optimal dough properties.

A direct approach to the determination of the relation of gluten content to extensogram area is afforded by the addition of either starch or gluten to a flour dough. This was done in the instance of two flours, with the results shown in Figure 20. The broken lines toward the right represent the doughs to which washed wet crude gluten was added, the solid lines to the left are doughs to which wet starch washed from the same flours was added, and the point of junction of the solid and broken lines indicates the protein content of the original flour.

It must be admitted that neither the wet starch nor the wet gluten thus employed in altering the protein content of the mixture can be regarded as having properties identical with the same substances in a natural flour. In other words, a dough thus brought to a certain level of gluten content might not be identical with a dough prepared from an unmanipulated flour of equivalent gluten content. The doughs involved in Figure 20 are significant, however, to the extent that they

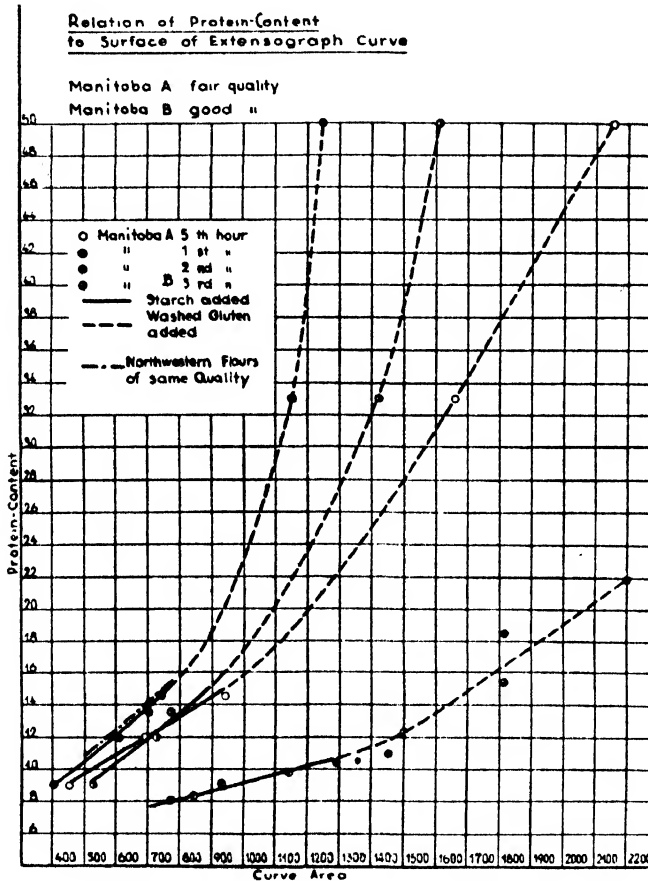


Fig. 20. Relation of protein content, as effected by dilution with starch or by reinforcement with washed gluten, to area under extensograms, and as a function of fermentation time.

undoubtedly represent trends. Moreover, the use of Northwestern flour in preparing a series of blends, as represented by the topmost curve at the lower left, yielded doughs possessed of properties very similar to those prepared by diluting with wet wheat starch.

When the data recorded in Figure 20 were combined with other observations upon normal flours of varying protein, the following relationships emerge:

Crude protein, %	Area under extensogram [optimal value]
8	600
10	800
12	1040
14	1270
16	1500

### Relationship of Fermentation Time and Mixing Time

The conclusion reached by Swanson and Working (1926) and others that an extension of the mixing operation beyond the optimum will tend to reduce the period of time in fermentation requisite to the production of bread of superior physical properties (disregarding flavor) finds support in these studies. Extensograms afford a convenient and useful index of optimum fermentation time, but it must be recognized that this time cannot be stated definitely in terms of minutes, since other variables in baking techniques exercise some effect. Instead, the expression should be in relative terms, with the understanding that the actual interval must be adapted to the formula, manipulation, type of bread desired, and other details of bake-shop practice.

### Fermentation Tolerance

This dough characteristic has been described by a committee of the American Association of Cereal Chemists (Cereal Chem. 11: 441) as "the range over which positive or neutral response is obtained." Objections might be raised to this definition because it includes an interval through which a *positive* response is obtained, which might imply a progressively *increasing* response through the interval up to a maximum and thence to the actual registration of a negative response. In actual baking practices fermentation tolerance is thought of as an interval through which the optimum properties are reached and *persist essentially unchanged*. In other words, it would not include an earlier period during which a positive response was progressively increasing, nor a subsequent interval during which the response was diminishing and approaching the "neutral" state.

While extensive data are not yet available to establish statistically the relation between extensograms and bake-shop experience, it does appear that the extensogram area will constitute a useful criterion of fermentation tolerance as thus defined.

### Stickiness of Doughs

It has been our experience that when flours require more than 68% of water (15% flour moisture basis) to yield doughs having a mobility equivalent to 500 farinograph units, the resulting doughs will tend to

be sticky or adhesive. Also the farinograph is useful in indicating the limits of mixing treatment before a dough becomes sticky.

### **Tolerance to the Action of Dough Dividers and Molders**

Baking technologists and bakery engineers are cognizant of variations in the tolerance of doughs to the vigorous action of dough dividers. In general, doughs which are short and "bucky" are most sensitive. Since these properties find expression in the oxynumber, calculated as indicated in the early part of this paper, it appears that flours with an oxynumber of 25 or less may be rated as sensitive to the action of dough dividers.

In operating the dough molder, two extremes of conditions may be encountered in widely varying doughs: (1) those represented by doughs which tend to draw together or decrease in length immediately following their discharge from the molder, due to a high modulus of elasticity, and (2) those which become too thick at the ends, due to too low viscosity, or too great an extensibility. Direct measurements of the rolls of dough one minute after their discharge from the molder of the extensograph may serve to disclose a tendency toward either of these extremes, and, indeed, these characteristics may prove to be predictable from the data taken from the extensogram.

### **Summary**

In the excited state, *i.e.*, during continuous dough mixing, the effect of oxidizing reagents upon physical dough properties is not registered significantly by a farinogram. In resting doughs, prepared either with or without yeast, the action of such reagents becomes evident when tests are made with the extensograph.

Properties of untreated doughs which can be determined from the extensogram appeared to be highly indicative of the direction and magnitude of the effect of such oxidizing reagents. Thus the "oxynumber" is computed from the formula:

$$\frac{\text{Area}}{F/E \times 10}$$

A low value approaching zero indicates a strongly negative reaction, a high value in excess of 50 means a strongly positive reaction, with gradations between passing through a neutral response between 30 and 40. The exact position of the neutral response number will be contingent upon dough formula, shop practice, and equipment.

The reciprocal of this oxynumber appears to be a linear function of the logarithm of the time of rest if tested in the prescribed manner, *i.e.*, 1, 2, 3, and 4 hours after mixing; in fact, the oxynumber plotted

against log. rest time approaches a straight line, but not as closely as does the relation first mentioned. The angle of departure of such curves from the horizontal affords a useful basis of estimating the rate and magnitude of the relative alterations in physical structure of the dough which occur with the lapse of time.

Treatment of flour with chlorine effected a substantial change in those physical properties which are registered in the  $F/E$  ratio of their doughs. The first increment of  $\text{Cl}_2$ , sufficient to reduce the pH of the dough to 5.4, effected the greatest change in this particular, and additional increments which increased the relative acidity of the dough progressively to pH 4.9 produced little additional change in  $F/E$  ratio. Area under the extensogram was greater in the flour treated to pH 5.4 and increased somewhat upon the addition of more chlorine to pH 4.9.

Papain substantially altered those dough properties which can be demonstrated by the extensograms, and when bromates were superimposed upon such doughs the action of the papain was restrained or reduced. The same tendencies were observed with cysteine and cysteine-bromate combinations.

Heat treatments of certain flour types appear to effect changes in dough properties analogous to those resulting from the use of bromates.

Diastatic malt flour added to doughs in normal proportions did not appear to affect those physical properties which are controlled by the protein matrix of the dough.

Area under the extensograms appears to afford a basis for prediction of bread-baking quality, values in excess of 1200 indicating large loaf volumes, areas below 400 very small loaf volumes, with gradations between these extremes.

Area under the extensogram is also related to gluten content when the gluten is of normal quality and the flour has been accorded proper conditioning or treatment with oxidizing reagents. Tentative relations between these two variables are: area =  $600 \pm$  for 8% crude protein; area = 1500 for 16% crude protein, with equivalent areas for intermediate protein levels.

Fermentation tolerance can be estimated from the relative change in extensogram area with the lapse of time. It must be recognized that the actual time in minutes through which dough maintains a fairly uniform optimum quality will also be a function of mixing, dough formula, type of bread desired, and other variables. Accordingly, the tolerance must be expressed in relative rather than definite intervals of time. The same is true, in general, of the estimation of optimum fermentation time from the curve area when the latter is plotted as a function of time of rest.

Tolerance to the action of dough dividers and dough molders can be predicted from an appropriate analysis of farinograms and extensograms.

#### Literature Cited

- Jørgensen, Holger  
1935 Über die Natur der Bromatwirkung. *Mühlenlab.* 5: 113-126.  
Munz, Emil, and Bailey, C. H.  
1936 Effect of enzymes of malted wheat flour upon certain properties of flour and dough. *Cereal Chem.* 14: 445-457.  
Swanson, C. O., and Working, E. B.  
1926 Mechanical modification of dough to make it possible to bake bread with fermentation only in the pan. *Cereal Chem.* 3: 65-83.

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## THE MOISTURE CONTENT AND GROWTH OF MOULD IN FLOUR, BRAN, AND MIDDINGS

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(Received for publication August 21, 1939)

It was shown by Kent-Jones and Amos (1930) and confirmed by Barton-Wright (1938) that bacterial numbers in flour decrease with time. Bacterial troubles are rarely found in mills, and when they do occur are generally of a specialized nature. For example, the sulphur bacterium, *Microspirum* sp., was found to be causing considerable trouble in one mill. In the bakehouse too, there is only one serious bacterial disease, that due to various strains of *B. mesentericus*, the causative agent of so-called "ropy" bread. On the other hand, moulds have long been known to cause spoilage in mill products, especially flour, bran, and middlings, often rendering them unfit for human and animal consumption.

The moisture content of a medium is one important factor influencing the growth of moulds and the present investigation is an analysis of this factor for the growth of moulds in flour, bran, and middlings.

When flour is exposed to air maintained at a constant relative humidity it loses or gains water until equilibrium is reached when the vapour pressure of water in the flour is equal to that of the air (Bailey, 1920, and Gane, 1938). If the air has a high relative humidity the flour not only takes up water but also becomes mouldy. Flour of high water content also becomes mouldy if stored in closed containers.

The relation between water content, relative humidity, and mould growth can be studied in two ways:

(1) Small samples can be exposed to constant temperature and relative humidity and the times noted for the appearance of mould. The water content at equilibrium can also be measured.

(2) The increase in mould content of large samples of flour of known water content stored at constant temperature in sealed containers can be followed.

The first method is probably more accurate for an investigation of this kind, because there is a marked tendency for flour of high moisture content to condition itself and increase its water content with progressive increase in fungal numbers. This increase in water content is possibly due to the water of respiration of the increasing mould population.

### Mould Growth on Flour Exposed to Constant Temperatures and Relative Humidities

Small samples (approximately 4 g.) of flour were spread on petri dishes 7 cm. in diameter which were supported on glass tripods above solutions of sodium chloride contained in museum jars with ground glass stoppers. The original water content of the flour was 14%. The jars were stored at 20°, 15°, 10°, and 5°C. Two samples were held at each of the relative humidities and temperatures. The flour was examined regularly and the time of appearance of moulds noted. The relation between the concentration of sodium chloride solution and the relative humidity maintained above it was as follows:

NaCl per 100 cc. water, g.	0	4	8	12	16	20	24	28	32
Relative humidity	100	97.5	95.1	92.6	90.2	87.7	85.1	82.5	79

These values were interpolated from a curve obtained by plotting available data of the vapour pressure of solutions of varying concentration. The relative humidity above solutions of sodium chloride is practically independent of temperature.

Table I and Figure 1 give the times for the appearance of mould.

TABLE I  
TIME IN DAYS FOR MOULD TO APPEAR ON FLOUR EXPOSED TO DIFFERENT  
RELATIVE HUMIDITIES AT CONSTANT TEMPERATURES

Temperature	Relative humidity (%)								
	100	97.5	95.1	92.6	90.2	87.7	85.1	82.5	79.0
20° C.	8	9	12	13	14	18	29	79	—
15°	11	11.5	15	18	23	40	57	—	—
10°	18	18.5	20	25	36	64	123	—	—
5°	20	25	27	43	53	123	—	—	—



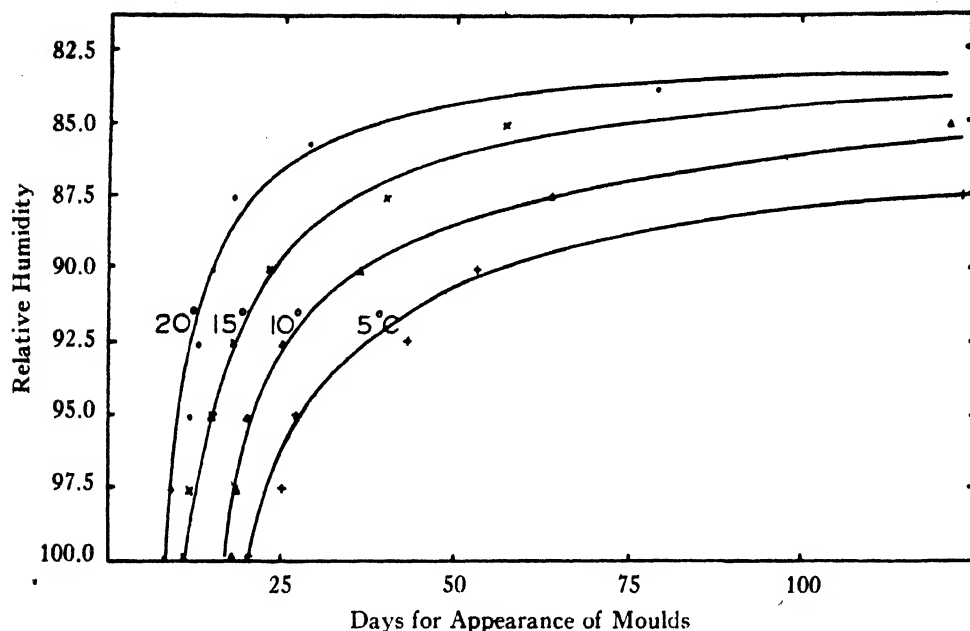


Fig. 1. Time for the appearance of mould on flour exposed to different relative humidities and temperatures.

The reciprocals of these times can be used to express the rate of onset of mouldiness (Table II and Figure 2).

TABLE II  
RELATIVE RATES OF THE APPEARANCE OF MOULDS ON FLOUR EXPOSED TO  
DIFFERENT RELATIVE HUMIDITIES AT DIFFERENT  
CONSTANT TEMPERATURES

Temperature	Relative humidity (%)							
	100	97.5	95.1	92.6	90.2	87.7	85.1	82.5
20° C.	125	111	83	77	71	55.5	34.5	12.5
15°	91	91	67	55.5	43	25	17.5	—
10°	55.5	55.5	50	40	27.7	15.8	8.5	—
5°	50	40	37	23.5	18.9	8	—	—

The effect of exposure of flour to air at high relative humidities is as follows:

(1) At constant temperature the time of appearance of mould decreases as the relative humidity is increased.

(2) At constant humidity the time for appearance of mould increases as temperature is decreased.

(3) The relative humidity to which air must be lowered to ensure that mould does not appear is lower at high than at low temperature.

In the example given (Fig. 1) mould growth was prevented when the

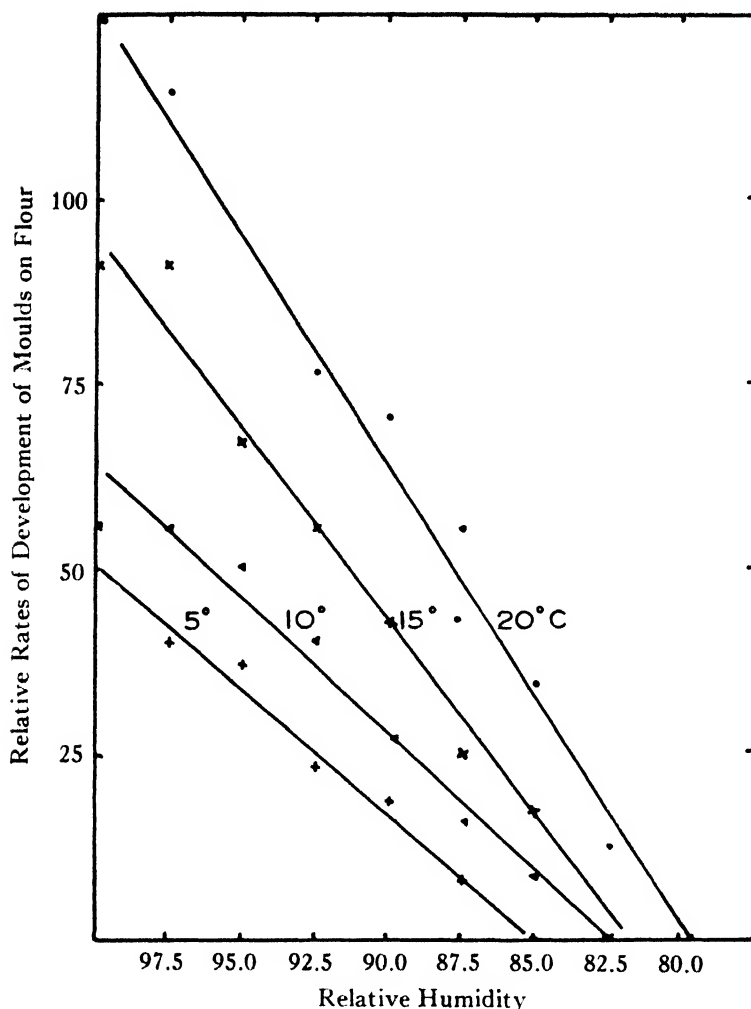


Fig. 2. Relative rate of development of moulds on flour exposed to different relative humidities and temperatures.

flour was exposed to a relative humidity of 79% at 20°C. (32% sodium chloride solution), to a relative humidity of 82.5% at 15°C. and 10°C. (28% sodium chloride solution), and to a relative humidity of 85% at 5°C. (24% sodium chloride solution).

These results with flour are similar to those applying to growth of moulds on thin films of nutrient agar (Tomkins, 1929). When samples of bran (original water content approximately 14%) were exposed above solutions of sodium chloride in the same way as flour, moulds made their appearance but at lower humidities.

There appear to be two possible explanations for mould growth occurring at lower humidities on bran than on flour, namely: (1) Bran is a "better" medium for growth. (2) The fungi generally associated

with bran are able to grow at lower humidities than those generally associated with flour.

It is difficult to define "good" or "better" as applied to media, but it has been observed (Tomkins, 1929) that growth takes place at a lower relative humidity on a film of nutrient medium than on a film of plain agar or on a glass surface. Similarly the composition of the medium may affect to some extent the range of humidity within which growth is possible.

TABLE III

TIME IN DAYS FOR THE APPEARANCE OF MOULD ON SAMPLES OF BRAN EXPOSED TO DIFFERENT RELATIVE HUMIDITIES AT 20° C.

Sample of bran	Relative humidity (%)				
	100	95.1	90.2	85.1	79
English bran	6	6	12	17	35
Mixed wheat bran	6	7	12	17	35
English middlings	6	7	10	17	35
Mixed middlings	6	6	9	17	35

The organisms mainly responsible for mouldiness in flour have not been examined in a sufficient number of instances to allow any definite statement to be made about their identity or frequency of occurrence. Flour exposed to humidities between 90% and 80% is attacked principally by *Penicillium* spp., bran exposed to the same humidities by *Penicillium* spp., and *Aspergillus* spp. It is not impossible that the latter forms are able to grow at a somewhat lower vapour pressure than the former.

Mould growth has not been observed on whole meal exposed above sulphuric acid of specific gravity 1.219 which maintains a relative humidity of 75% at 20°C. Moulds have, however, been observed to appear on bran after it had been exposed for 3 to 6 months to a relative humidity of 75%.

### The Rapid Determination of the Water Content of Flour Necessary to Allow the Growth of Mould

The experiments described above suggest that the point to which the water content of flour must be adjusted to prevent the growth of moulds can be obtained by exposing thin layers to a 32% solution of sodium chloride (relative humidity 80%) or in the case of bran to sulphuric acid of specific gravity 1.219 (relative humidity 75%) at 20°C. for three days to allow equilibrium to be established and then determining their water content.<sup>1</sup>

<sup>1</sup> A saturated solution of sodium chloride (relative humidity 76%) could be used instead, since a difference of 1% in relative humidity is within the experimental error of the method.

The water content of flour at which mould growth does and does not occur is given in Table IV.

Duplicate samples of flour were exposed to 24%, 28%, and 32% solutions of sodium chloride at 20°C. (relative humidities 85, 82.5, and 79%) and their water contents determined by exposing 2 g. on a 7-cm. petri dish to 120°C. for one hour. The water content is expressed in two ways, *i.e.* (a) as a percentage of original weight and (b) as g. water per 100 g. dry flour.

TABLE IV

WATER CONTENT OF FLOUR IN EQUILIBRIUM WITH 85, 82.5, 79% RELATIVE HUMIDITY AT 20° C.

(a) % water content

(b) g. water per 100 g. dry flour

Sample of flour	Water content					
	Over 24% salt; 85% relative humidity		Over 28% salt; 82.5% relative humidity		Over 32% salt; 79% relative humidity	
	Mould after 40 days (a)	(b)	Mould after 50 days (a)	(b)	No mould after 3 months (a)	(b)
Patent	16.8 17.4	20.2 21.0	16.8 16.8	20.1 20.2	16.3 15.95	19.4 19.0
Manitoba patent	16.9 16.8	20.4 20.1	15.9 16.3	18.95 19.5	15.6 14.9	18.5 17.5
Patent	16.9 17.0	20.3 20.5	16.1 16.1	19.2 19.2	15.6 15.5	18.4 18.3
Long patent	18.0 17.8	22.0 21.7	17.0 16.9	20.6 20.4	16.3 16.4	19.5 19.6
English straight-run	17.9 17.8	21.8 21.5	16.6 17.0	19.8 20.5	16.2 16.5	19.4 19.8
Low grade	17.4 17.6	21.1 21.2	16.5 16.5	19.5 19.8	15.9 15.9	18.9 19.0
Average	17.36	20.98	16.54	19.81	15.92	18.94

The various samples differ slightly in their water contents in equilibrium with a constant relative humidity. It is therefore impossible to fix with precision for all flours the critical water content above which mould growth is possible and below which it is impossible.

Broadly speaking it can be said that most samples containing more than 16% of water are just sufficiently moist to permit of mould growth, whereas samples containing less than 16% of water are sufficiently dry to prevent mould growth.

Samples of bran were exposed at 20°C. to a 32% solution of sodium chloride (relative humidity 79%), sulphuric acid of specific gravity 1.191 (relative humidity 80%) and specific gravity 1.219 (relative humidity 75%). The water contents of these samples are given in Table V.

TABLE V  
WATER CONTENT OF SAMPLES OF BRAN EXPOSED ABOVE 32% SALT AND  
SULPHURIC ACID SOLUTION

(a) g. water per 100 g. of moist flour  
(b) g. water per 100 g. of dry flour

Sample of bran	Water content					
	32% salt (79% R.H.)		H <sub>2</sub> SO <sub>4</sub> specific gravity 1.191 (80% R.H.)		H <sub>2</sub> SO <sub>4</sub> specific gravity 1.219 (75% R.H.)	
	Mould after 40 days		Mould after 40 days		No mould	
	(a)	(b)	(a)	(b)	(a)	(b)
Ground wheat meal	15.9	18.9	16.5	19.8	14.9	17.5
	16.1	19.2	16.15	19.2	14.8	17.3
Mixed wheat meal	16.5	19.7	16.6	19.9	15.6	18.5
	17.8	21.7	16.8	20.2	15.4	18.2
Mixed wheat bran	17.0	20.5	18.2	22.4	15.4	18.5
	17.0	20.5	18.0	21.3	14.8	17.3
English wheat bran	17.9	21.6	18.2	22.1	15.4	18.2
	17.6	21.5	17.4	21.5	15.4	18.3
Straight bran	16.9	20.4	17.6	21.5	15.2	18.0
	16.4	20.0	17.1	20.7	15.2	17.9
Bran B	17.0	20.5	18.1	22.1	16.0	19.0
	16.8	20.3	16.8	20.2	15.6	18.4
Average	16.9	20.4	17.3	20.9	15.3	18.1

Some variation is again noted in the percentage water content of the various samples in equilibrium with the same relative humidity.

Whole meal of 17.0% water content was observed to become mouldy while whole meal of 15.3% water content remained free from mould. Bran of 17% water content became mouldy after 40 days and once bran of 15.3% water content became mouldy after 90 to 120 days.

#### Growth of Mould on Flour of Different Water Contents Stored in Closed Containers

Experiments were also set up with flour of different moisture contents stored in closed containers at 20°, 15°, and 10°C. The samples at each moisture content were in triplicate, and the number of fungal

spores per gram was estimated by the method described by Barton-Wright (1938). The water contents of the samples at the different temperatures are given in Table VI.

TABLE VI  
WATER CONTENT OF SAMPLES

Temperature	Percentage water content of flour				
20° C.	19.6	17.0	16.5	15.0	14.0
15° C.	19.6	—	16.5	15.0	—
10° C.	19.6	—	16.5	15.0	—

The average number of spores per gram of flour at the start of the experiment was 3,200 to 5,400. The most detailed work was carried out on the samples stored at 20°C., since these, as was to be expected, were the first to show increases in fungal numbers. Tables VII, VIII, and IX show the results for the three temperatures, whilst the logarithms of the fungal numbers for 20°C. are plotted in Figure 3.

TABLE VII  
FUNGAL SPORES PER GRAM OF FLOUR AT DIFFERENT WATER CONTENTS, STORAGE AT 20° C. IN CLOSED CONTAINERS

Days	Water content, %				
	19.6	17.0	16.5	15.0	14.0
—	3,200	4,800	3,600	5,200	5,400
10	168,000	5,900	3,700	5,400	6,000
17	5,000,000	6,600	3,600	5,700	—
24	24,000,000	38,900	—	—	—
31	37,000,000	1,000,000	—	—	—
40	30,000,000	5,000,000	3,300	5,300	5,100
75	—	—	34,000	—	—
145	—	—	100,000	5,600	5,300
250	—	—	54,000	3,000	2,900

TABLE VIII  
FUNGAL SPORES PER GRAM OF FLOUR AT DIFFERENT WATER CONTENTS, STORAGE AT 15° C. IN CLOSED CONTAINERS

Days	Water content, %		
	19.6	16.5	15.0
—	3,200	3,600	5,200
10	10,000	—	—
17	174,000	—	—
24	4,800,000	—	—
31	12,000,000	—	—
40	32,000,000	3,200	5,400
145	—	18,400	—
250	—	38,000	3,300

TABLE IX

FUNGAL SPORES PER GRAM OF FLOUR AT DIFFERENT WATER CONTENTS, STORAGE AT 10° C. IN CLOSED CONTAINERS

Days	Water content, %		
	19.6	16.5	15.0
—	3,200	3,600	5,200
10	3,600	—	—
17	5,300	—	—
24	10,400	—	—
31	63,000	—	—
40	6,150,000	3,600	5,400
250	—	3,200	—

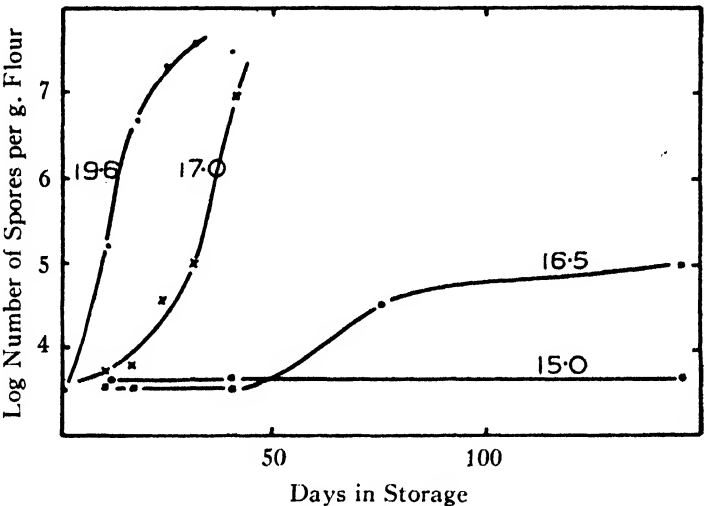


Fig. 3. Spore content of flour of 19.6, 17.0, 16.5, and 15.0% water content stored at 20° C.

On the whole these figures agree well with those obtained by the method of exposing flour to atmospheres of different relative humidities and support the conclusion that as far as flour is concerned the critical water content for fungal growth to take place is approximately 16%. In commercial practice, however, this figure would be near the danger point; for safety a water content of 15% or less and storage at relative humidities of not more than 80% R.H. are advisable to eliminate mould growth.

Discussion

The relations between incidence of mouldiness and the water content of flour are similar to those found for the growth of fungi on thin films in equilibrium with known relative humidities. The growth of some fungi, e.g., *Trichoderma* and *Botrytis*, is not possible on films

exposed to humidities below 90% relative humidity; the growth of others, *e.g.* *Rhizopus*, is checked on films exposed to a relative humidity of 86% whilst others again, *e.g.* *Aspergillus niger*, have been observed to show growth at a relative humidity of 80%, but not below this value (Tomkins, 1929, and unpublished results). Groom and Panisset (1933) found that the germination of spores of *Penicillium chrysogenum* was checked below a relative humidity of 81% but report the mildewing of book materials at 72.6% relative humidity. Galloway (1934) observed that some mould fungi which attack textiles ceased to grow if the relative humidity were reduced to 85% to 90% but that certain species of *Aspergillus* grew at relative humidities of 75% to 80%. Thom and Le Fevre (1921) investigated the flora of corn meal. No bacterial activity was noted in meals of commerce. *Aspergillus repens* only was found to grow in meal of water content of 13% to 15% while several species grew if the water were 16% or more.

Koehler (1938) studied fungus growth on shelled corn as affected by moisture. He obtained constant humidities by passing a current of air through saturated solutions of different salts or by exposing his samples above the solutions in closed containers. He found that certain fungi grew only when the water content was equivalent to 90% relative humidity and that *Penicillia* did not cease to grow until the water content was reduced to 15.5% and *Aspergillus glaucus* until the water content was reduced to 14.5%. Above this figure growth took place, but ceased below it.

The nature of the chemical changes brought about in flour by growth of moulds is being investigated.

### Summary

The growth of moulds in flour has been investigated from the viewpoint of temperature, relative humidity of the air, and water content of the flour.

The critical water content for flour at which mould growth ceases at all temperatures of storage can be determined by exposing a thin layer of flour to constant weight over a 32% solution of sodium chloride (32 g. salt per 100 cc. of water) at 20°C. For bran the corresponding solution is a saturated solution of the salt.

### Literature Cited

- Bailey, C. H.  
1920 The hygroscopic moisture of flour exposed to atmospheres of different relative humidity. *Ind. Eng. Chem.* 12: 1102-1104.
- Barton-Wright, E. C.  
1928 Studies on the storage of wheaten flour, III. Changes in the flora and the fats and the influence of these changes on gluten character. *Cereal Chem.* 15: 521-541.



- Galloway, L. D.  
1934 The moisture requirements of mould fungi, with special reference to mildew in textiles. Shirley Inst. Memoirs **13**: 27.
- Gane, R.  
1938 Water relations of flour. F. I. B. Ann. Rep. (in press).
- Groom, P., and Panisset, T.  
1933 Studies on *Penicillium chrysogenum* Thom in relation to temperature and relative humidity of the air. Ann. Appl. Biol. **20**: 633-660.
- Kent-Jones, D. W., and Amos, A. J.  
1930 Preliminary studies in the bacteriology of wheat and flour. Analyst **55**: 248.
- Koehler, B.  
1938 Fungus growth in shelled corn as affected by moisture. J. Agr. Res. **56**: 291-307.
- Thom, C., and Le Fevre, E.  
1921 Flora of corn meal. J. Agr. Res. **22**: 179-188.
- Tomkins, R. G.  
1929 Studies of the growth of moulds. Proc. Roy. Soc. **B105**: 375.

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## THE USE OF SOYBEAN BETA-AMYLASE TO FOLLOW THE MODIFICATION OF STARCH<sup>1</sup>

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(Received for publication November 21, 1939)

Iwanoff, Kurgatnikov, and Kirsanova (1937) reported that they have established fine differences between individual starches by application of diastatic methods. It was previously noted by Stone (1896), O'Sullivan (1904), Nagao (1911), Sherman, Walker, and Caldwell (1919), Amberger (1921), Hermano and Rask (1926), Kashiwaya (1930), and others that differences occur in the digestibility of starches from different sources. The mechanical or chemical treatment which a starch receives also affects the susceptibility of the starch to amylolytic digestion. Malloch (1929) and Karacsonyi and Bailey (1930) showed that overgrinding of flours results in increased diastatic activity. Blish (1937) reported that grinding wheat starch in a ball mill increased the amount of susceptible starch. Raw wheat starches finely pulverized in a rod mill were easily hydrolyzed by both alpha- and beta-amylase according to Stamberg and Bailey (1939). Wiegel's (1933) discussion of C. F. Lintner's work emphasizes the effect of pre-treatment temperature of the starch paste on the viscosity and on the extent of digestion by diastase. The efforts of Mangels (1926), Andrews and Bailey (1934), Snider and Coleman (1937), Sallans and Anderson (1937), Redfern and Johnston (1938), and others were also directed toward determining the effect of substrate on amylolytic digestion.

<sup>1</sup> Journal Paper No. J-696 of the Iowa Agricultural Experiment Station, Ames, Iowa. Project No. 517. Supported in part by a grant from the Corn Industries Research Foundation.

Martin and Newton (1938), using the beta-amylase from soybeans, have shown that the temperature at which the starch substrate is prepared affects the rate as well as the degree of digestibility. The digestion curves showed characteristic differences which were striking enough to suggest the application of the method to industrial modifications of starches.

In its essentials the method consists in heating each of a series of starch suspensions at a different temperature for 30 minutes, then digesting the resulting paste for three hours at 40°C. with the soybean enzyme. A determination of the reducing value at the end of this time allows plotting the yield of maltose in percent against the temperature of pretreatment of the starch. The characteristic curves thus obtained differ in their extrapolated intercept on the temperature axis, in their slope, and in their value for maximum digestion. The effects of heat, mechanical modification, oxidation, acid-hydrolysis, and of some dry-milling processes on corn starch are reported in order to demonstrate the application of the method to measuring changes in the susceptibility of the starch granules to digestion by soybean beta-amylase. It seems probable that soybean powder could be used in place of the soybean amylase concentrate.

### Experimental

*Preparation of enzyme suspension.*—A soybean amylase concentrate was prepared and its activity determined according to the method of Newton and Naylor (1939). The concentrate was stored at 5°C. and the activity checked at regular intervals. The concentrate used was capable of producing 875 mg. of maltose per mg. of concentrate at 40°C. in 30 minutes, using a soluble potato starch as substrate and buffered at pH 4.7 with phosphate buffers. This concentrate had zero dextrinogenic activity (Ohlsson and Edfeldt, 1933). Forty mg. of the concentrate was suspended in 50 ml. of ice-cold distilled water and kept in an ice bath at all times. Exactly 5 ml. of the suspension was used for each digestion. Fresh enzyme suspensions were prepared daily.

*Preparation of substrate.*—Two grams of the dry starch were weighed into a 100-ml. volumetric flask, 0.2 ml. of 0.2M  $\text{Na}_2\text{HPO}_4$  and 9.8 ml. of 0.2M  $\text{NaH}_2\text{PO}_4$  added to buffer the solution at pH 4.7, the mixture diluted to about 90 ml., thoroughly shaken to disintegrate any lumps, and placed in a constant temperature water bath (at the desired temperature) for exactly 30 minutes. The suspension was then rapidly cooled to approximately 40°C., diluted to a volume of 100 ml. at this temperature, and allowed to reach the exact temperature of the digestion bath (40°C.). After thorough mixing, two 5-ml. samples were withdrawn for the blank reducing sugar determination.

*Digestion.*—Exactly 5 ml. of the enzyme suspension was then added to the starch suspension in the digestion bath, mixed, and the digestion allowed to proceed for three hours. The flask was shaken at half-hour intervals during the digestion. At the end of three hours the contents of the flask were thoroughly mixed and two 5-ml. samples withdrawn for the sugar determination. The reducing equivalent of the blank and of the digestion mixture was determined by the potentiometric method of Martin and Newton (1938). The potentiometer can be dispensed with in routine work because of the color change of the solution which occurs 0.05 ml. before the potentiometric end-point.

### Discussion of Digestion Curves

*Unmodified starches.*—The digestion curves of the starches of rice, tapioca, wheat, potato, and corn have been published by Martin and Newton (1938). Additional supplementary determinations have been made to determine the extrapolated intercepts on the temperature axis. It is understood that the beginning of digestion for many of the starches was not as sudden as the graphs show but was more gradual and often took place over a range of several degrees. In determining the intercepts these rounded portions at the beginning of the curves were not considered. The intercepts were evaluated by extrapolation of the straight-line portions of the digestion curves. The slopes were also determined from these straight-line portions. Table I shows the

TABLE I  
DIFFERENT PLANT STARCHES

Kind of starch	Optimum temperature for preparing substrate (°C.)	Slope	Extrapolated intercept on temperature axis
Rice	80 or above	5.3	64
Tapioca	80	4.0	58
Wheat	90	2.3	54
Potato	70	9.1	56
Corn	90	3.5	59
Sweet potato	80	2.5	54
Waxy sorghum	100	5.0	67
Waxy maize	80	6.2	63

optimum temperature for the preparation of the starch substrate, the extrapolated intercept on the temperature axis, and the slope. Sweet-potato starch and waxy sorghum starch have been added to the list. Notable differences appear in the slopes and intercepts.

In Figures 1 and 2 are presented the digestion curves of seven unmodified corn starches and the curve for waxy sorghum starch.

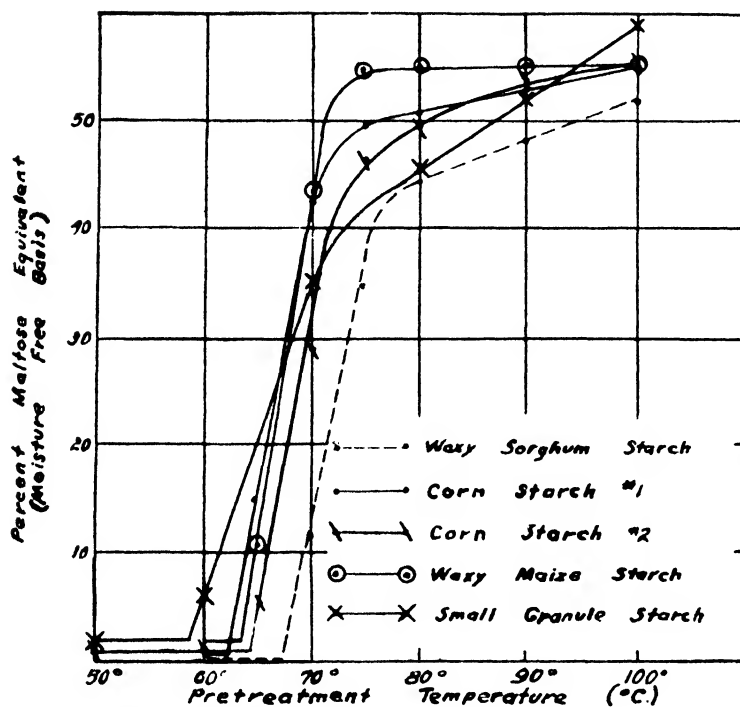


Fig. 1. Unmodified starches.

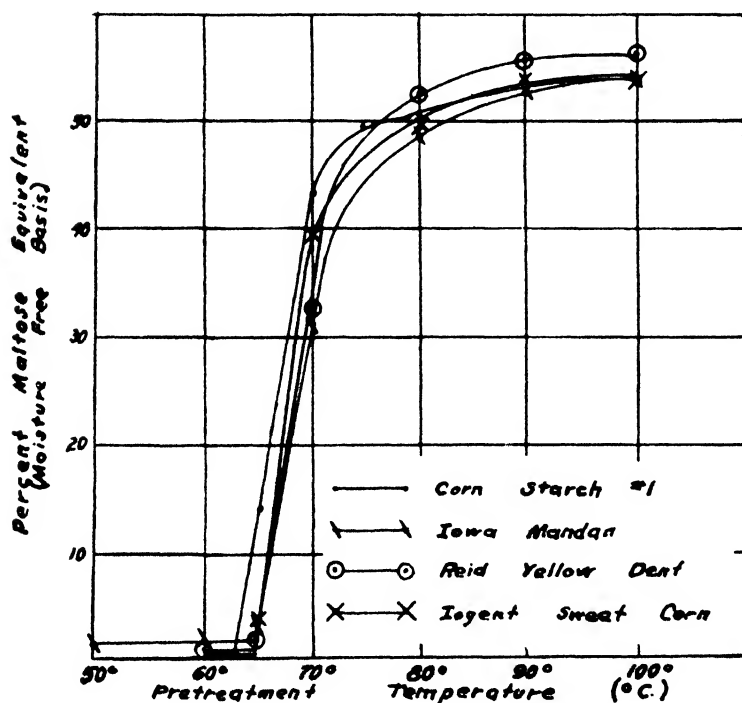


Fig. 2. Unmodified corn starches.

This allows a comparison of the two waxy starches as well as of the corn starches. At pretreatment temperatures below 65°C. the small-granule starch is the most susceptible one to digestion by soybean beta-amylase, but above 65°C. is replaced by waxy maize starch. The digestion of the small-granule starch below 65°C. is abnormal for unmodified corn starches. The small-granule starch again gives abnormal results between 75° and 90°C., at this stage being less digestible than the other unmodified corn starches. This is probably due to the higher gelatinization temperature of the small granules. Sample No. 1 of commercial "pearl" starch was more susceptible to digestion than sample No. 2 of commercial "pearl" starch at pretreatment temperatures up to 90°C. When gelatinized at 90° to 100°C. all of the unmodified corn starches except the small granule starch were digested to about the same extent.

*Correlation of amylolytic digestion with loss of birefringence.*—An investigation to determine the meaning of the intercept of the enzyme digestion curve disclosed no correlation between the temperature of maximum gelatinization and the intercept on the digestion curve. The available data did show some correlation of the temperature of maximum gelatinization with the temperature at the mid-point of the steep portion of the digestion curve.

Huss (1923) reported that Congo Red stained swollen and ruptured granules of starch but not the intact granules. He correlated the percentage of stained granules with the gelatinization temperature in an approximate way. Experiments in this laboratory showed that the staining began to take place at approximately the same temperature at which the polarization cross began to disappear and that both effects corresponded closely to the beginning of digestion by the beta-amylase of soybeans, *i.e.*, to the intercept of the digestion curve.

The following method was devised for obtaining curves which would show the loss of birefringence (disappearance of the polarization cross) as the temperature was raised:

One tenth of a gram of starch was weighed into a test tube containing 5 ml. of distilled water. The tube and contents were heated with occasional stirring for thirty minutes in a constant-temperature bath at the desired temperature. The sample was allowed to cool to room temperature and was then shaken to give a uniform suspension. A small drop of the suspension was placed on a microscope slide, diluted with a drop of water, and mixed well. A cover slip was placed over the drop and, when the counting required much time, sealed with vaseline. The total number of granules in ten or twelve fields and also the number showing a polarization cross were counted, using the most convenient power of the microscope. The percentage of granules showing no birefringence was calculated and plotted against the pretreatment temperature of the starch.

In Figure 3 a few curves showing loss of birefringence are compared with the digestion curves of the same starches. In each case thus far

examined the birefringence curve was very similar to the enzyme digestion curve. Different methods of counting the birefringent granules gave different results. When using nicol prisms a certain value was obtained by counting all granules showing any birefringence whatever. When polaroid lenses were used it was expedient to eliminate those granules which were only partially gelatinized and consequently only partially birefringent and to count only those granules possessing a definite cross. Since the values could vary between these two extremes, the correlation of the digestion curves with the birefringence curves was not as good as might be expected. Nevertheless, these

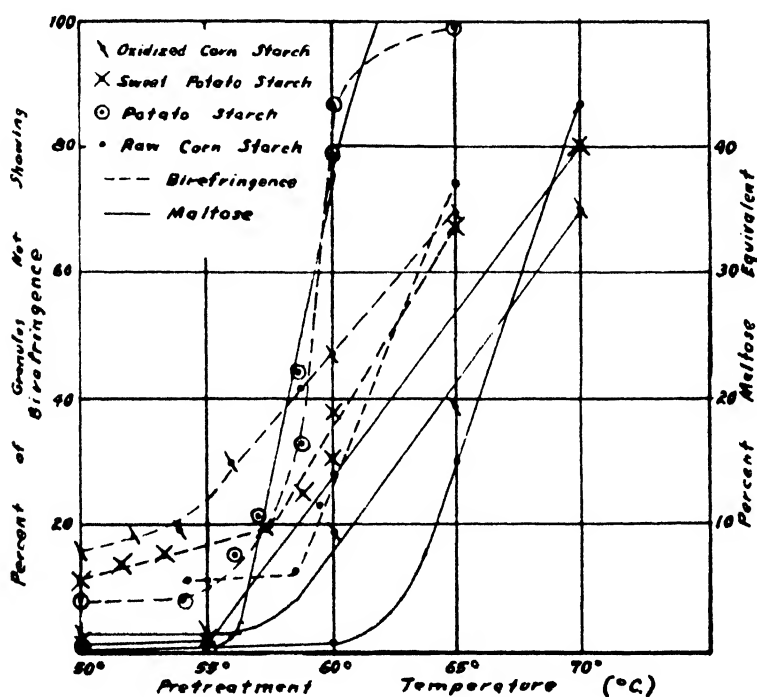


Fig. 3. Correlation of amyolytic digestion with loss of birefringence.

curves indicate that soybean beta-amylase begins to digest starch at approximately the same pretreatment temperature at which the starch begins to lose its birefringence and begins to gelatinize. As more and more of the starch granules lose their double refraction and gelatinize the rate of digestion increases as a straight-line function, giving the steepest portion of the digestion curve. When the majority of the more heat-labile starch granules have been gelatinized the digestion and birefringence curves bend toward zero slope as the more heat-resistant granules gelatinize. According to Alsberg (1938) the gelatinization temperature of starch granules depends on size and surface area, follow-

ing the laws of surface tension. His explanation is supported in part by the curve for the small granule starch (Fig. 1), but it seems that other factors are also involved.

*Mechanically modified corn starches.*—Figure 4 presents the curves of the amylolytic digestion of starches ground in a ball mill for 150, 400, and 600 hours. Birefringence gradually disappeared on grinding, with the result that the starch ground for 600 hours contained only an occasional doubly refractive granule. The reducing values (expressed in the  $R_{cu}$  units of Richardson, Higginbotham, and Farrow, 1936) varied from 4.0 on the 150-hour sample to 24.2 on the 600-hour sample.

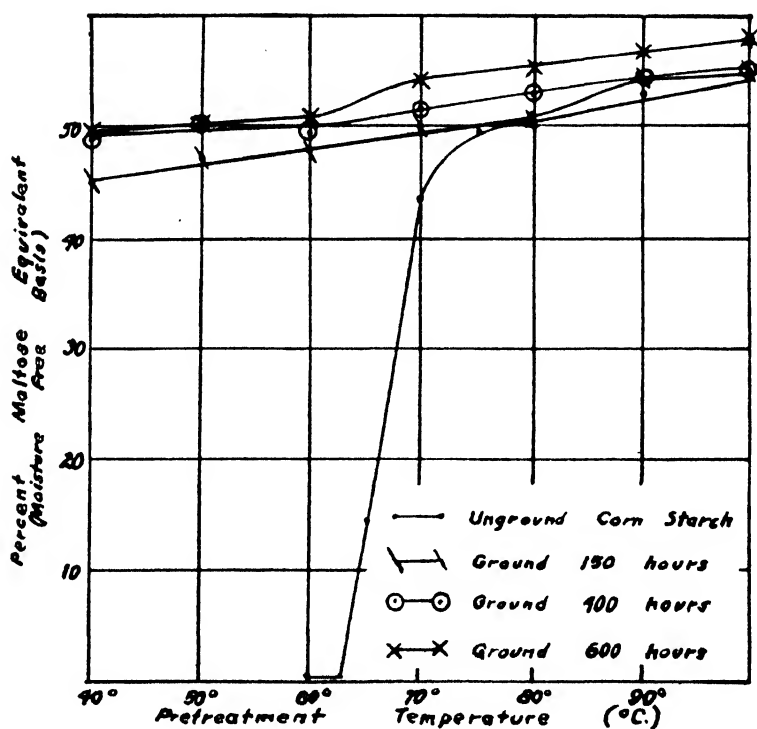


Fig. 4. Ground corn starches.

It is evident from the curves that the grinding process has increased the susceptibility of the starch to enzyme action. This increase in susceptibility is most noticeable at the lower pretreatment temperatures of 40, 50, and 60°C. The digestible starch at these pretreatment temperatures has been increased from less than 1% (raw starch) to more than 45% by grinding for 150 hours. On the other hand when the starch substrates are heated at 100°C., grinding causes very little increase in the amount of digestible starch over that available from raw starch.

*Oxidized corn starches.*—The oxidized (so-called “chlorinated”) starches were prepared by treatment of raw corn starch with alkaline hypochlorite produced electrolytically. Starches oxidized to varying degrees were digested by the beta-amylase of soybeans. Figure 5 shows the curves obtained. Oxidation with small amounts of chlorine (up to 0.05 equivalent of chlorine per glucose unit) caused only a slight retardation of the enzyme digestion at all pretreatment temperatures. Oxidation with 0.1 equivalent or more of chlorine impeded digestion by soybean beta-amylase at higher pretreatment temperatures and reduced the total maltose equivalent producible. With the use of

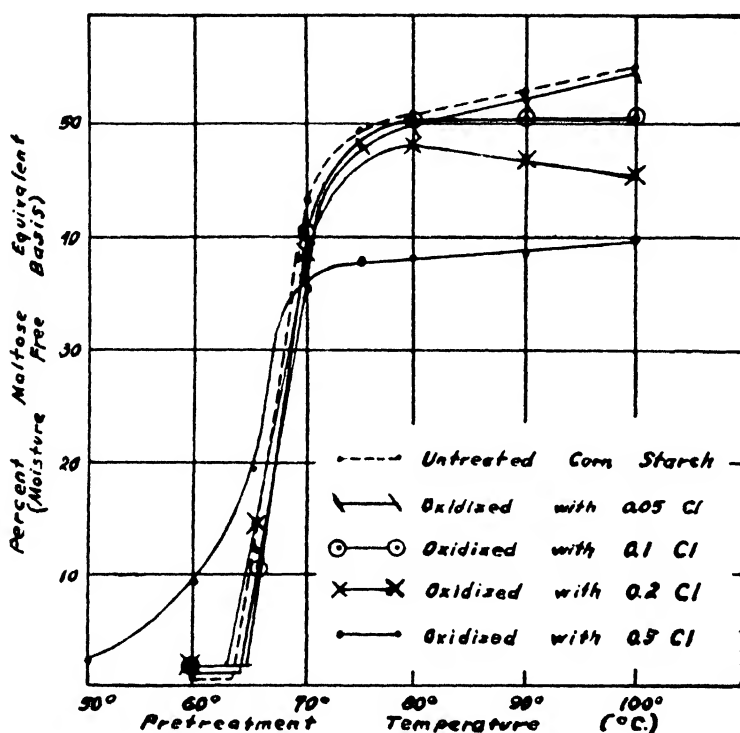


Fig. 5. Oxidized corn starches.

larger amounts of chlorine (0.5 equivalent) a marked increase in the digestion at lower pretreatment temperatures was observed in addition to the above-mentioned effect. This was further demonstrated by the digestion curves of two commercially chlorinated starches. These two curves were very similar to the curve of the starch oxidized with 0.5 equivalent of chlorine. The logical conclusion of this study on oxidized starch is that oxidation changes some of the glucose units of starch to structures which are not attacked by beta-amylase under the conditions of these experiments. In support of this observation,



previous experiments showed that gelatinized corn starch oxidized more drastically than the above failed to show any digestion by malt extract.

*Dextrins and acid-hydrolyzed starches.*—Dextrins are modified starches produced by heat with or without the addition of chemicals or by enzymes. Three samples of the cold-water-insoluble fraction of commercial dextrins were subjected to the action of soybean beta-amylase digestion and the resulting values plotted in Figure 6. The  $R_{cu}$  values for these dextrins were as follows: No. 1, 30.7; No. 2, 71.3; and No. 3, 108.9. Thus the order of degradation from the most to the

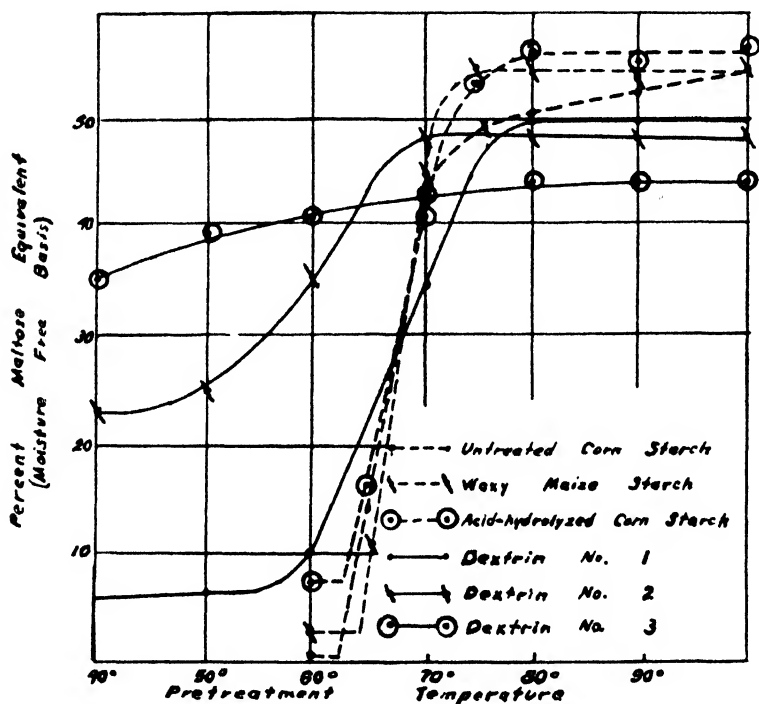


Fig. 6. Dextrins and acid-hydrolyzed starches.

least degraded sample as measured by copper reduction was No. 3, No. 2, and No. 1. This order of degradation was also definitely illustrated by the digestion with soybean beta-amylase. Increased dextrinization has increased digestion at low pretreatment temperatures and has decreased digestion at high pretreatment temperatures. Dextrins from which the soluble fraction has not been removed would be expected to show digestion curves similar to those of the ground starches.

Figure 6 also contains the digestion curve of a soluble starch prepared according to Gore (1928) by treating corn starch with 13% HCl

for 20 hours at room temperature. The action of amylase on this substrate followed very closely the corresponding action on waxy maize starch. The raw corn starch substrates prepared at 80°C. produced about 50% maltose in three hours, whereas waxy maize starch and this sample of soluble starch produced about 55% maltose under the same treatment. Two "thin-boiling" starches prepared commercially with acid catalysts gave results similar to those of the Gore starch. Slight acid hydrolysis generally increases digestibility at high pretreatment temperatures but has little or no effect at low pretreatment temperatures.

*Corn flours and brewers' flakes.*—Both corn flours and brewers' flakes are made by tempering corn grits with moisture and rolling on hot rolls. Figure 7 presents the digestion curves of three samples of

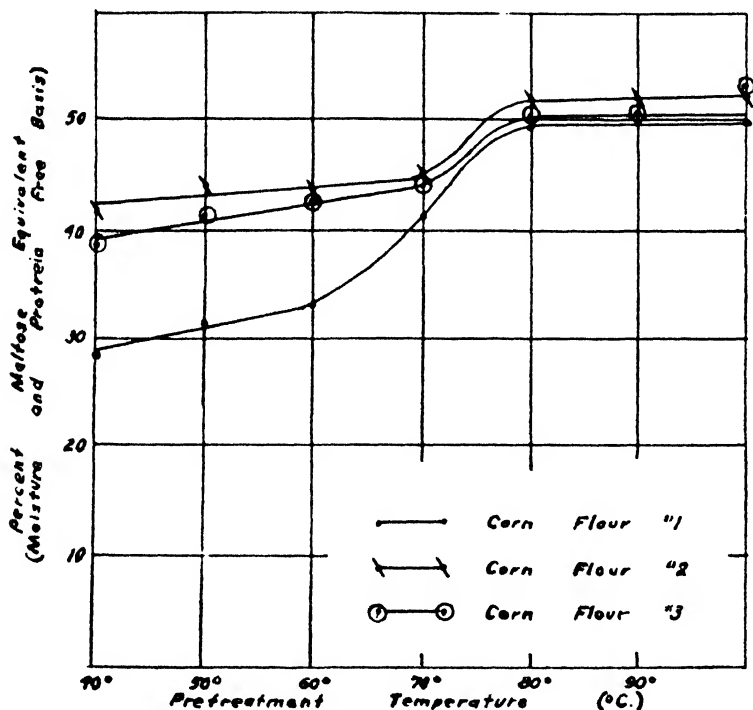


Fig. 7. Corn flours.

commercial corn flour. As measured by amylolytic hydrolysis the order of increasing degradation was No. 1, No. 3, No. 2, with the variation between samples No. 2 and No. 3 being almost within the range of experimental error.

By comparison with Figure 4 it is evident that the corn flours behaved like ground starches. By variations in the procedures of tempering and flaking, the starch in the corn flours apparently has been

ruptured considerably though not as much as the corn starch ground for 150 hours in the ball mill. The increased digestion between 70° and 80° due to gelatinization of previously nonsusceptible granules is further evidence that the starch in these corn flours is not modified as much as that ground in the ball mill.

Two samples of commercial paste flakes were also characterized by soybean amylase digestion. The curves obtained were not plotted here but both of them corresponded very closely to the curve for corn flour No. 3 as shown in Figure 7. No natural amylase was present in these flakes nor was there any in the corn flours. The curves, therefore, represent digestion by the added soybean beta-amylase and are not complicated by the factors of autolysis which were found to change the character of the curves in the digestion of brewers' flakes.

Four samples of brewers' flakes were ground and digested by soybean amylase in the usual manner. The upper curves of Figure 8 were obtained. Each of these curves showed a minimum amount of digestion at a certain temperature. These minima fell between 50° and 75°C. and indicated the presence in the brewers' flakes of a natural corn amylase which had not been destroyed in the milling process. Autodigestions gave the results shown by the dashed curves in Figure 8. The abnormally high autodigestion value shown by sample No. 2 was

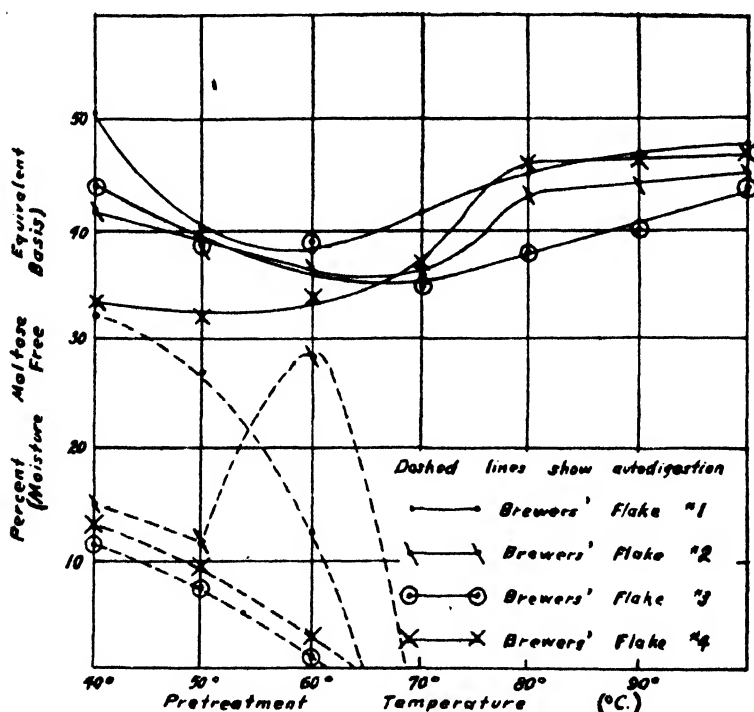


Fig. 8. Brewers' flakes.

checked by three independent determinations with an accuracy of  $\pm 1.0\%$ . No evidence is available to indicate whether this is due to activation of the enzyme by heat or to increased susceptibility of the starch at this temperature. The curves show the variation which can be expected in such commercial samples.

Bulbrook (1928) has found that the natural amylase in corn is principally of the alpha type. Thus the autodigestion of the brewers' flakes may be due to action of alpha-amylase in the flakes. The digestion curves of Figure 8 seem to indicate a sum of the combined actions of alpha- and beta-amylase. The alpha-amylase acts during the pretreatment up to the temperature at which it is destroyed; with all pretreated starches, the beta-amylase acts (in addition to any alpha-amylase) during digestion. On this basis, an approximate measure of the maltose which soybean beta-amylase would produce if no corn amylase were present could be obtained by subtracting the autodigestion values from the total digestion values. Further work is in progress, using other samples of brewers' flakes.

### Summary

The method of Martin and Newton (1938), which uses soybean beta-amylase to digest starch suspensions prepared at different temperatures, has been employed to measure the susceptibility of different starches to digestion and to measure the effect of various treatments on this susceptibility.

A microscopic study of several starches indicated in each case that the starch granules became digestible at approximately the same temperature at which they began to lose their birefringence and began to gelatinize.

Characteristic digestion curves have been presented to show the changes in susceptibility of starch when ground in a ball mill for varying lengths of time, when oxidized by different amounts of chlorine, when dextrinized or acid-hydrolyzed to different degrees, and when subjected to the dry-milling processes by which corn grits are converted to corn flours, paste flakes, and brewers' flakes.

### Acknowledgments

The authors wish to express their thanks to Dr. R. M. Hixon for constant advice and cooperation and also to the following concerns for the donation of materials: American Maize Products Company, Clinton Company, Keever Starch Company, Miller Cereal Mills, Penick and Ford, Ltd., and Stein, Hall and Company, Inc.

### Literature Cited

- Alsberg, C. L.  
1938 Structure of the starch granule. *Plant Physiol.* **13**: 295-330.  
Amberger, K.  
1921 Nachweis fremder Stärke im Getreidemehl. *Z. Untersuch. Nahr. Genuss.* **42**: 181-182.

- Andrews, J. S., and Bailey, C. H.  
1934 The amylases of normal and germinated wheat flours. *Cereal Chem.* 11: 551-556.
- Blish, M. J.  
1937 Proceedings of the twenty-third annual meeting of the American Association of Cereal Chemists, p. 13.
- Bulbrook, H. J.  
1928 Preparation and properties of the amylase of maize. Thesis. Iowa State College Library, Ames, Iowa.
- Gore, H. C.  
1928 A new soluble starch and an improved polarimetric Lintner method. *Ind. Eng. Chem.* 20: 865-866.
- Hermano, A. J., and Rask, O. S.  
1926 A consideration of certain reactions of starches with special reference to enzyme hydrolysis. *Cereal Chem.* 3: 361-392.
- Huss, H.  
1923 Untersuchungen über die Quellung der Stärkekörner. *Arkiv Botanik* 18 (8): 1-23.
- Iwanoff, N. N., Kurgatnikov, M. M., and Kirsanova, V. A.  
1937 Über die durch die diastatische Methode festgestellte Verschiedenheit in der Struktur der Stärke. *Enzymologia* 4: 163-168.
- Karacsonyi, L. P., and Bailey, C. H.  
1930 Relation of the overgrinding of flour to dough fermentation. *Cereal Chem.* 7: 571-587.
- Kashiwaya, G.  
1930 Starches. I. Starches from various species of plants. *J. Chem. Soc. Japan* 51: 719-729; *Chem. Abstr.* 26: 618 (1932).
- Malloch, J. G.  
1929 Studies on the resistance of wheat starch to diastatic action. *Can. J. Research* 1: 111-147.
- Mangels, C. E.  
1926 Factors affecting the diastatic activity of wheat flour. *Cereal Chem.* 3: 316-322.
- Martin, V. D., and Newton, J. M.  
1938 Comparative rates of amylase action on starches. *Cereal Chem.* 15: 456-462.
- Nagao, Y.  
1911 Vergleichende Studien über die Einwirkung von Pankreasdiastase auf Hafer- und Weizenstärke. *Z. Exp. Path. Ther.* 9: 227-237.
- Newton, J. M., and Naylor, N. M.  
1939 Soybean amylase. I. The concentration and characterization of soybean amylase. *Cereal Chem.* 16: 71-78.
- Ohlsson, E., and Edfeldt, O.  
1933 Amylasen in ruhenden und keimenden Samen. III. Hafer. *Z. physiol. Chem.* 221: 174-179.
- O'Sullivan, J.  
1904 A comparison of the products of the hydrolysis of potato starch with those obtained from cereal starches. *J. Chem. Soc.* 85: 616-623.
- Redfern, S., and Johnston, W. R.  
1938 The effect of the substrate on diastatic activity. *Cereal Chem.* 15: 327-341.
- Richardson, W. A., Higginbotham, R. S., and Farrow, F. D.  
1936 The reducing power and average molecular chain-length of starch and its hydrolysis products, and the constitution of their aqueous pastes. *J. Text. Inst.* 27: T 131-157.
- Sallans, H. R., and Anderson, J. A.  
1937 Sources of error in the determination of the diastatic power of malt. *Cereal Chem.* 14: 708-720.
- Sherman, H. C., Walker, F., and Caldwell, M. L.  
1919 Action of enzymes upon starches of different origin. *J. Am. Chem. Soc.* 41: 1123-1129.

- Snider, S. R., and Coleman, D. A.  
1937 Soluble starch: A study of its properties with a recommended supplementary procedure for determining its suitability for use in the Lintner determination. *Cereal Chem.* 14: 1-17.
- Stamberg, O. E., and Bailey, C. H.  
1939 Studies on wheat starch. II. The action of amylases on raw wheat starches. *Cereal Chem.* 16: 319-330.
- Stone, W. C.  
1896 The carbohydrates of wheat, maize, flour and bread and the action of enzymic ferments upon starches of different origin. U. S. D. A. Off. Exp. Sta. Bul. 34.
- Wiegel, E.  
1933 Verlauf der Warmverkleisterung von Kartoffelstärke insbesondere bei verschiedener thermischer Vorgeschichte. *Kolloid-Z.* 62: 310-324.

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## THE WHEAT-MEAL-TIME-FERMENTATION TEST. IV. INHIBITORY AND ACCELERATING FACTORS<sup>1</sup>

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(Received for publication November 16, 1939)

In previous papers (Swanson, 1937; Swanson and Dines, 1939; and Swanson, 1939) it was shown that various factors influence the "time" or the interval between the moment the doughball, of either wheat meal or flour, is put into the water and the moment a break can be observed on the under surface at the dough-water interface. The following main facts were shown: The time on flours is longer than on meals and the time on flours from wheats which have a short time is usually as long as on flours from wheats which have a long time. Adding the bran or shorts which were removed in milling does not shorten the time on flour, but makes it longer. The protease pepsin and the protease activator, commercial cysteine used in the form of the monohydrochloride, decreased the time. These two substances, to a certain extent, overcame the lengthening effect of bran and shorts. A few trials indicated that extracting bran with water did not affect its lengthening effect, but the water extract shortened the time. A few trials also indicated that the wheat germ decreased the time. It was also shown that protease inhibitors, notably  $\text{KBrO}_3$  and commercial Cebione or ascorbic acid, increased the time. The investigations thus far indicate that the time is influenced by three groups of factors besides the gluten quality: (1) proteases, (2) protease activators, and (3) protease inhibitors. The effects of the various substances were greater on long-time than on short-time wheats and, hence, substances can be added which will shorten the time of long-time wheat meal, so that it is no

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<sup>1</sup> Contribution No. 65, Department of Milling Industry.

<sup>2</sup> Credit is due Glen West, student assistant, for painstaking work in performing the laboratory manipulations.

longer than that of a short-time meal, and substances can also be added which will make the time of short-time meals as long as those of the long. Some substances which lengthen the time on flour are in the bran and some substances which shorten the time are in the germ.

The investigations discussed in this paper were concerned first with the question of whether various treatments of bran and germ would influence their effects on time. Along with these there were several supplementary experiments.

Tenmarq was used as a representative of a long-time wheat and Blackhull and Chiefkan as short-time wheats. The time on the meal of Tenmarq was 103 minutes, of Blackhull 52, and of Chiefkan 42. The time on the flours were: Tenmarq 106, Blackhull 120, and Chiefkan 104 minutes. It should be remembered that there is always obtained on samples from every wheat or flour considerable variation in time, but these figures were the basis for selecting these varieties and in several instances were used as checks. A 2,000-gram sample of each of these wheats was milled into flour, bran, and shorts. Some wheat of each variety was reserved for making tests on the meals. These trials were not performed in the order they are presented but were subsequently arranged for as logical treatment as possible. Chiefkan was used in a few trials because the sample of Blackhull was exhausted.

### Exchanging Bran and Shorts

Since the time on Blackhull flour was found to be as long as that on the Tenmarq flour, would there be any difference in the effects of these respective brans if exchanged on the flours? To ascertain this the bran from each wheat was used on its own flour and on the flour from the other wheat. Shorts were also included because they contain more of the germ and also more of the endosperm than the bran. The figures obtained in this exchange are found in Table I.

TABLE I  
EFFECT OF EXCHANGING BRAN AND SHORTS

	Tenmarq flour	Blackhull flour
	<i>Min.</i>	<i>Min.</i>
<i>Tenmarq bran or shorts</i>		
15 g. flour (check)	106	120
12.6 g. flour + 2.4 g. bran	153	130
13.0 g. flour + 2.0 g. shorts	155	117
10.6 g. flour + 2.4 g. bran + 2 g. shorts	119	71
<i>Blackhull bran or shorts</i>		
12.6 g. flour + 2.4 g. bran	169	108
13.0 g. flour + 2.0 g. shorts	161	115
10.6 g. flour + 2.4 g. bran + 2 g. shorts	148	69

Bran and shorts when added separately produced a lengthening effect on Tenmarq flour but not consistently on Blackhull flour. When both were used together there was an increase in time on Tenmarq flour, but on Blackhull flour there was a notable decrease in time. This decrease on Blackhull was produced with the bran plus the shorts from both wheats and may have been due to the germ in the shorts. Why this should affect the Blackhull flour and not the Tenmarq flour is not clear. However, there is no evidence in these figures that the effects of Tenmarq bran were in any way different from Blackhull bran.

### Effects of Heating the Bran

Several trials (literature cited) as well as figures in Table I had established that untreated bran would increase the time on flour. To determine if heating the bran would have any effect, trials were made with heated bran, water-extracted heated bran, and extract of heated bran. Heating would destroy any enzymes present and the water extraction might remove the inhibiting substance. One-hundred-gram portions of bran from Tenmarq and from Blackhull were heated at 100°C. for about 2½ hours. Portions of these heated brans were ground in a hammer mill to pass a ½-mm. sieve and then used with the two flours as shown in Table II.

TABLE II  
EFFECTS OF HEATED BRAN ON THE "TIME" OF FLOURS

	Tenmarq flour	Blackhull flour
	<i>Min.</i>	<i>Min.</i>
<i>Tenmarq heated bran</i>		
15 g. flour (check)	103	106
14 g. flour + 1 g. bran	170	162
13 g. flour + 2 g. bran	204	148
12 g. flour + 3 g. bran	227	166
<i>Blackhull heated bran</i>		
15 g. flour (check)	98	107
14 g. flour + 1 g. bran	181	166
13 g. flour + 2 g. bran	200+	175
12 g. flour + 3 g. bran	200+	173

The heated brans produced an increase in the time of both flours but there is no evidence to show that the effect of Tenmarq bran was any different from that of Blackhull even after heating.

### Effects of the Extracts from Heated Bran

To 25-gram portions of each of the heated brans was added 125 cc. of water and the material was allowed to soak over night. These were then placed on linen cloth such as is used for filtering crude fiber and as



much liquid squeezed out as practicable. This liquid was then centrifuged and portions of the clear liquid were used on the two flours as indicated in Table III. Since 125 cc. of water was added to 25 g. of bran, each cc. of the extract represented  $\frac{1}{5}$  g. of bran. Because of the large water absorptive capacity of bran, it was not practicable to obtain a liquid by this method which would represent as large portions of bran as were used in the trials shown in Tables I and II.

TABLE III  
EFFECTS OF WATER EXTRACTS OF HEATED BRAN

	Tenmarq flour	Blackhull flour
	<i>Min.</i>	<i>Min.</i>
<i>Tenmarq heated bran extract</i>		
15 g. flour (check)	104	107
15 g. flour + 2 cc. = $\frac{2}{5}$ g. bran	117	116
15 g. flour + 4 cc. = $\frac{4}{5}$ g. bran	115	114
15 g. flour + 8 cc. = $1\frac{3}{5}$ g. bran	132	133
<i>Blackhull heated bran extract</i>		
15 g. flour (check)	101	107
15 g. flour + 2 cc. = $\frac{2}{5}$ g. bran	115	115
15 g. flour + 4 cc. = $\frac{4}{5}$ g. bran	112	120
15 g. flour + 8 cc. = $1\frac{3}{5}$ g. bran	136	139

The effects of the extracts of heated bran were much smaller than that of the bran material. The figures show that these extracts produced no decrease in time but a small increase and also that there was no difference between the extracts of the two brans. In a previous trial (literature cited) there was an indication that the water extract of unheated bran produced a small decrease in time.

#### Effect of Water-Extracted Heated Bran

Portions of the heated brans were soaked in 400 cc. of water over night. They were then placed on linen and washed several times with water, after which they were spread out in thin layers till air-dry. After grinding in a hammer mill they were used in the trials presented in Table IV. It is evident that the extraction with water did not remove any of the inhibiting substances nor was there any difference between the effects of two water-extracted brans.

#### Effect of Pre-soaking the Bran

Ground bran of Tenmarq and of Blackhull were placed in beakers using the weighed portions desired for use in the doughballs, and 9 cc. of water was added. The beakers were then covered with watch glasses

TABLE IV  
EFFECTS OF WATER-EXTRACTED HEATED BRAN

	Tenmarq flour	Blackhull flour
	<i>Min.</i>	<i>Min.</i>
<i>Tenmarq water-extracted heated bran</i>		
15 g. flour (check)	103	111
14 g. flour + 1 g. bran	187	209
13 g. flour + 2 g. bran	248	251
12 g. flour + 3 g. bran	300+	250+
<i>Blackhull water-extracted heated bran</i>		
15 g. flour (check)	103	111
14 g. flour + 1 g. bran	181	227
13 g. flour + 2 g. bran	260+	217+
12 g. flour + 3 g. bran	275+	275+

and kept in the fermentation cabinet at 30°C. for 4 hours. Doughballs were then made by adding the weighed portions of the flours and of the meals from Blackhull and Tenmarq as shown in Table V. Pre-soaking should allow any inhibiting or activating substance in the bran to be liberated and thus become more active. The results show, however, that the effects of the presoaking were insignificant.

TABLE V  
EFFECT OF PRESOAKED BRAN ON FLOUR AND MEAL

Amt. flour or meal	Amt. bran	Tenmarq		Blackhull	
		Flour	Meal	Flour	Meal
		<i>Min.</i>	<i>Min.</i>	<i>Min.</i>	<i>Min.</i>
15 g. (check)		112	165	133	60
14 g. + 1 g. Tenmarq presoaked bran		182	172	192	74
13 g. + 2 g. Tenmarq presoaked bran		227	177	173	115
12 g. + 3 g. Tenmarq presoaked bran		236	216	212	135
14 g. + 1 g. Blackhull presoaked bran		193	167	193	83
13 g. + 2 g. Blackhull presoaked bran		239	197	185	130
12 g. + 3 g. Blackhull presoaked bran		264	222	216	187

### Effect of Mixing Starch with the Meal so as to Attenuate the Gluten Structure

One question in connection with the use of bran mixed into the doughball is the attenuating effect on the gluten. This by itself should have weakened the gluten structure and as a consequence shortened the time. On the contrary the bran lengthened the time and the lengthening was somewhat greater with the larger amounts of bran. Thus there is some substance in the bran which slows down the

reactions which cause the break on the doughball water-interface. Wheat starch would not have this inhibiting substance and for comparison it was mixed with flour in varying proportions so as to attenuate the gluten network. The proportions used and results obtained are given in Table VI.

TABLE VI  
EFFECT OF DILUTING FLOUR WITH STARCH

Proportions in mixture		Tenmarq	Blackhull
Flour	Starch	<i>Min.</i>	<i>Min.</i>
g.	g.		
15	0	108	130
13	2	118	130
11	4	105	144
9	6	101	116
7	8	105	97

There was no shortening of time on Tenmarq flour beyond the experimental error. On the Blackhull there was no decrease in time until the proportion of starch was a little more than half the flour. The effect of starch is in contrast to the effect of bran which would also dilute the gluten structure and this should shorten the time. Apparently weakening the gluten structure does not have a shortening effect until the proportion of the attenuating substance is large. This indicates that the lengthening effect of bran is due to an inhibiting substance.

Trials were also made by diluting the wheat meal with starch. The results obtained are given in Table VII.

TABLE VII  
EFFECT OF DILUTING WHEAT MEAL WITH STARCH

Proportions in mixture	Tenmarq	Chiefkan
	<i>Min.</i>	<i>Min.</i>
15 g. meal + 0 g. starch	123	52
13 g. meal + 2 g. starch	90	41
11 g. meal + 4 g. starch	61	49
9 g. meal + 6 g. starch	64	49
7 g. meal + 8 g. starch	59	52

The variations in the figures for Chiefkan meal are within the experimental error and hence it may be said that diluting its gluten structure with starch had no effect. The shortening effect of 2 g. and 4 g. of starch is quite evident on the Tenmarq meal and this is probably due to the weakening of the gluten structure. However, the 6 g. and 8 g. do not seem to have any further additive effect.

### Effects of Commercial Wheat Germ

In a previous trial (literature cited) it was shown that the presence of a considerable amount of wheat germ obtained either by adding a mill stream rich in this substance or adding the germ ends of the kernels would shorten the time. This suggested that further trials be made with the yellow flaked germ obtained in flour mills. The germ was first ground to pass a  $\frac{1}{2}$ -mm. sieve. Trials were then made by mixing the ground germ with flour and meal. Water extract of the germ was used in varying amounts. A few trials were also made with the water-extracted germ. The extracting with water was done in the same manner as with the bran. The respective amounts used and the results obtained from these trials are given in Table VIII. The addition of

TABLE VIII  
EFFECT OF COMMERCIAL WHEAT GERM

	Tenmarq	Blackhull
	<i>Min.</i>	<i>Min.</i>
Meal (check)	103	52
Meal + 1 g. germ	62	46
Meal + 2 g. germ	50	47
Meal + 3 g. germ	45	49
Flour (check)	106	120
Flour + 1 g. germ	126	126
Flour + 2 g. germ	64	62
Flour + 3 g. germ	52	57
Flour + extract of germ 1 cc.	73	78
Flour + extract of germ 2 cc.	62	73
Flour + extract of germ 4 cc.	58	66
Flour + extract of germ 6 cc.	58	73
Flour + extract of germ 8 cc.	54	70
Flour + extracted germ 1 g.	86	98
Flour + extracted germ 3 g.	120	112

germ and water extract of germ had a shortening effect on both the meals and the flours. In this respect the effects of the germ are in sharp contrast to the effects of the bran. That the active substance in germ is water-soluble is indicated by the effects of the germ extracts and by none or only small effects of the water-extracted germ.

### Effect of Heating the Germ

If the shortening effects of the germ are due to an enzyme, then this should be destroyed by heating. About 50 grams of the germ were heated at 100°C. for several hours and then ground in a hammer mill. Portions of this germ were mixed with the flours and then used in making doughballs. This heated germ had a peculiar effect on the

doughballs. It was not possible to get a definite end point. The balls flattened out and remained floating. Strings of dough developed on the under side, but no pronounced point at which disintegration started could be recorded. (These observations indicate that the heating of the germ produces some substance which has a pronounced effect on the physical properties of the doughball.)

A portion of the ground germ was extracted with water by letting it soak for several hours, then filtering and centrifuging. Portions of this water extract of heated germ were then used in making doughballs with the flours. It was difficult to get a satisfactory end point because the balls became stringy. The results as observed are given in Table IX and they show that the accelerating substance in the germ was destroyed by heating since there was no shortening of time as with the water extract of unheated germ.

TABLE IX  
EFFECT OF WATER EXTRACT OF HEATED GERM

	Tenmarq flour	Blackhull flour
	<i>Min.</i>	<i>Min.</i>
15 g. flour (check)	95	96
15 g. flour + 2 cc. = $\frac{1}{2}$ g. germ	109	117
15 g. flour + 4 cc. = 1 g. germ	120	112
15 g. flour + 8 cc. = 2 g. germ	119	116

### Effects of Conditions of Germination

The germ, as the center of the processes which start a new plant, would be rich in storage enzymes. In the process of germination still more enzymes would be developed by the epithelial layer at the boundary between the germ and the endosperm. The process of germination requires a moisture content of about 35% or more, and high moisture content may of itself cause changes in the wheat kernel so as to affect the time. To obtain this comparison one set of wheat samples were soaked in water at 60°C. and another set at 27°C. After soaking until the kernels became soft, both sets were kept moist at room temperatures for 1, 2, 3, and 4 days, respectively. At the end of each period the wheat samples were spread in thin layers to permit rapid air-drying and then stored in a dry place until ground for the tests. The samples soaked at 60°C. did not show any signs of germination, while the others showed increased amounts of sprouting with the progress of time. The ground meal from the sample germinated four days was the one mixed with meal of unheated wheat. The results of these trials are shown in Table X.

TABLE X  
EFFECTS OF SOAKING AT 27°C. AND 60°C. AND THE PROCESS OF GERMINATION

	Tenmarq		Chiefkan	
	60°C.	27°C.	60°C.	27°C.
	<i>Min.</i>	<i>Min.</i>	<i>Min.</i>	<i>Min.</i>
Check sample		121		59
14 g. meal + 1 g. germinated meal		150+		36
13 g. meal + 2 g. germinated meal		88		28
Kept moist for:				
1 day		53		32
2 days	34	29	29	23
3 days	43	20	29	21
4 days	34	20	29	17

It is apparent that simply soaking the wheat in water at 60°C. decreased the time, but there was no effect from the duration of the time of keeping the wheat moist. In the samples which were treated at 27°C. and in which germination took place there was a progressive decrease in time. After the samples had been germinated there was no essential difference between the time of the long Tenmarq and the short Chiefkan. In supplementary trial it was found that soaking the wheat one hour at 70°C. reduced the time of Tenmarq to 37 minutes and Blackhull to 35 minutes.

### Effects of Conditions of Storage

Wheat is continually undergoing change and this change is the more rapid the higher the moistures and the higher the temperatures. It was desired to learn what effects these conditions have on the doughball time. It was shown by Swanson (1937) that time on ground meal tends to increase with the duration of storage. In order to determine if the conditions of wheat storage have a notable influence on time of wheat, samples were treated as follows: Water was added to various portions so as to make the moisture contents 12%, 16%, and 20%, respectively. The 12% samples may be considered as the checks. After wetting, the wheat was placed in closed 4-ounce screw-cap bottles to minimize the moisture loss and also duplicate conditions in deep bins. One-half the bottles were stored in a room kept at 60°-65°F. and the other half on a laboratory shelf where the temperature ranged considerably higher. At the end of 1, 7, 28, and 84 days portions were ground and the time tests made with the results given in Table XI.

The results obtained on the 12% moisture samples show the range in experimental error when tests are far apart. Storing at 20% moisture increased the time for the two longer periods of storage.

This may have been due in part to the mellowing effects which caused a finer granulation. In a previous trial (Swanson, 1937) it was shown that finer granulation caused a longer time. The results show in particular that the condition of storage must vary considerably before there is any important influence on time.

TABLE XI  
EFFECTS OF STORING UNDER VARYING MOISTURES AND TEMPERATURES

Period of storage	Moisture content	Stored in laboratory		Stored at 60°-65°F.	
		Tenmarq	Chiefkan	Tenmarq	Chiefkan
<i>Days</i>	<i>%</i>	<i>Min.</i>	<i>Min.</i>	<i>Min.</i>	<i>Min.</i>
1	12	138	40	130	45
7	12	113	39	112	35
28	12	124	42	119	38
64	12	116	50	109	47
1	16	129	48	121	43
7	16	111	40	111	44
28	16	117	51	132	43
64	16	127	62	140	55
1	20	159	53	156	67
7	20		48	105	40
28	20	146	98	162	79
64	20	158	97	212	66

### Effects of Diastase

The process of germination would increase not only the proteases but also the diastases. Flour from germinated wheat or malted wheat flour is added to increase the diastatic activity of the flour and hence have a consequent effect on the rate and amount of gas production in the baking processes. To learn what effect these enzymes had on the doughball time, trials were made by mixing commercial diastase with both the meals and the flours. The results obtained are given in Table XII.

TABLE XII  
EFFECTS OF DIASTASE

	Tenmarq	Blackhull
	<i>Min.</i>	<i>Min.</i>
Meal + 5 mg. diastase	113	59
Meal + 10 mg. diastase	111	57
Flour + 5 mg. diastase	112	111
Flour + 10 mg. diastase	110	110

Additional data on the effects of diastase are given in connection with other trials which will be described later. The figures show that the diastase did not shorten the time of the meal or the flour. Hence, the effect of the 2 g. meal from germinated wheat was apparently not due to the diastase but rather to the protease (Table X).

### Effects of Malted Wheat Flour

The malted wheat flour used was a commercial product sold to mills that desire to blend in certain amounts in order to increase the diastatic activity of the flour whenever it is lower than demanded by the baker. In one set of determinations the malted wheat flour was first mixed with the 15 g. of meal when the water and yeast were added in making the three doughballs. In another set of determinations the malted wheat flour was mixed with the water on which the doughball was floated. For comparison pepsin was dissolved in the water on which the doughballs were floated. The results obtained are given in Table XIII.

TABLE XIII  
EFFECT OF MALTED WHEAT FLOUR AND PEPSIN

	Tenmarq	Chiefkan
	Min.	Min.
<i>Malted wheat flour incorporated with the doughball</i>		
Meal (check)	139	44
Flour (check)	157	104
Meal + 5 mg. M. W. F.	144	41
Meal + 10 mg. M. W. F.	153	43
Meal + 25 g. M. W. F.	156	42
Meal + 50 g. M. W. F.	177	45
Meal + 100 g. M. W. F.	176	47
Flour + 10 g. M. W. F.	126	115
Flour + 100 g. M. W. F.	133	116
<i>Malted wheat flour suspended in the water</i>		
Meal + .1 g. M. W. F.		42
Meal + .2 g. M. W. F.	184	44
Meal + .5 g. M. W. F.	227	44
Meal + 1.0 g. M. W. F.	240 +	43
Meal + 2.0 g. M. W. F.	240 +	41
Flour + .2 g. M. W. F.	143	104
Flour + 2.0 g. M. W. F.	123	102
<i>Pepsin dissolved in the water</i>		
Meal + 10 mg. pepsin	41	36
Meal + 20 mg. pepsin	40	39

On Tenmarq the malted wheat flour increased the time both when mixed with the doughball and when suspended in the water so as to act at the dough-water interface. On Chiefkan the time was not in-



fluenced. In contrast with the diastase, the pepsin had a marked effect in decreasing the time. Thus any protease which may be present in malted wheat flour has apparently no effect on time.

### Effects of Varying the Amounts of Yeast and Sugar

The addition of diastase or of malted wheat flour should stimulate yeast activity because of the availability of more yeast food. The amounts of yeast used in the time determinations are based on baking formulas, but sugar is omitted as a rule. To determine whether adding sugar in varying amounts and also using larger amounts of yeast would influence the results, the quantities of yeast and sugar as indicated in Table XIV were used.

TABLE XIV  
EFFECTS OF INCREASING AMOUNTS OF SUGAR AND YEAST

	Amt. sugar	Meal—Amt. yeast			Flour—Amt. yeast		
		2%	4%	6%	2%	4%	6%
	%	<i>Min.</i>	<i>Min.</i>	<i>Min.</i>	<i>Min.</i>	<i>Min.</i>	<i>Min.</i>
Tenmarq	0	134	117	102	166	122	103
	2	175	124	113	157	89	62
	4	185	153	125	169	92	63
	6	198	156	127	167	93	64
	8	186	152	159	171	100	65
Blackhull	0	79	60	53	200	156	122
	2	79	63	55	180	102	79
	4	88	69	75	173	102	80
	6	89	78	91	173	106	78
	8	96	83	89	178	105	77

The results obtained by varying the amounts of sugar and yeast show the following:

1. The time on the Tenmarq meal is longer in every instance than the time on Blackhull meal, the ratio being nearly 2 : 1. The time on Blackhull flour is longer than the time on Tenmarq flour, but the differences between the flours are small in comparison with the differences obtained on the meals.

2. When the yeast was increased the time was decreased in every instance but one. The duration of decrease was greater on the Tenmarq than on the Blackhull meal but the decreases were in the same degree on both flours.

3. When the sugar was increased the time was increased on the meal, and the more sugar the longer was the time. On the flour there was a distinct decrease in time with 2% sugar when 4% and 6% yeast was used. Increasing the sugar beyond 2% produced no further de-

crease with the larger amounts of yeast. With 2% yeast the time was decreased on the Blackhull flour when the sugar was increased beyond 2% but not on the Tenmarq flour.

4. The time differences between Blackhull meal and Tenmarq meal persisted with all the variations of the amounts of yeast and sugar. The time differences with the flours are smaller, and the time for Blackhull flour was longer in every instance.

5. There thus appears to be a real difference in the behavior of these two meals and flours which is not obliterated by varying either the amount of yeast or sugar.

### Effect of Inhibitors in Connection with Pepsin

In former trials (literature cited) it was shown that  $\text{KBrO}_3$  and Cebione increased the time and that pepsin decreased the time. Further trials were made with pepsin both alone and in combinations with the inhibitors Cebione and  $\text{KBrO}_3$ . These inhibitors were also used in combination with germ and predigested meal. Pepsin was also used alone on both flour and meal from long- and short-time wheats. The results of using pepsin both alone and in combination with Cebione or  $\text{KBrO}_3$  are given in Table XV.

TABLE XV  
EFFECTS OF PEPSIN IN COMBINATION WITH  $\text{KBrO}_3$  OR CEBIONE

$\text{KBrO}_3$	Cebione	Pepsin	Tenmarq meal	Blackhull meal
Mg.	Mg.	Mg.	Min.	Min.
0	0	0	103	52
0	0	2	53	35
0	$\frac{1}{2}$	0	207	133
0	1	0	227	151
0	2	0	238	194
$\frac{1}{2}$	0	0	159	72
1	0	0	192	82
2	0	0	236	98
0	$\frac{1}{2}$	2	85	52
0	1	2	89	54
0	2	2	97	58
$\frac{1}{2}$	0	2	56	38
1	0	2	60	40
2	0	2	66	42

In every case the  $\text{KBrO}_3$  and the Cebione when used by themselves increased the time, but when used in combination with pepsin their inhibiting effects were rendered either nil or much reduced. In some supplementary trials it was found that these effects also depended upon the relative quantities used. That is, one or the other might be present in such amounts as to neutralize each other.

### Effect of Germ in Connection with Inhibitors

That commercial wheat germ shortens the time was shown in figures given in Table VIII. If this shortening is due to a protease, then the germ should tend to neutralize the effects of  $\text{KBrO}_3$  and Cebione. That this did take place is shown in Table XVI using ground germ with both meal and flour.

TABLE XVI  
EFFECT OF WHEAT GERM IN COMBINATION WITH  $\text{KBrO}_3$  OR CEBIONE

	Tenmarq	Blackhull
	<i>Min.</i>	<i>Min.</i>
Meal alone (check)	98	52
Meal + 2 mg. $\text{KBrO}_3$	236	98
Meal + 2 mg. Cebione	238	194
Meal + 2 g. germ + 2 mg. $\text{KBrO}_3$	61	43
Meal + 2 g. germ + 2 mg. Cebione	50	48
Flour alone (check)	96	109
Flour + 2 mg. $\text{KBrO}_3$	133	116
Flour + 2 mg. Cebione	117	121
Flour + 2 g. germ + 2 mg. $\text{KBrO}_3$	86	55
Flour + 2 g. germ + 2 mg. Cebione	108	65

The germ overcame the inhibiting effects except with Cebione on the Tenmarq flour. This may have been because the quantity of the germ was not sufficient to overcome the inhibiting effects of the 2 mg. of Cebione.

### Influence of Predigesting the Meal

Several trials (unpublished data) have shown that water-and-flour dough undergoes marked changes in physical properties if simply allowed to stay in the dough-fermentation cabinet. It has also been shown in the preceding pages that additions of proteases, activators, and inhibitors have a great influence on time. What effect would simply staying in the cabinet have on time? The plan was to allow the doughball to predigest for four hours before the yeast was mixed in and then determine the effect of this predigestion on time. In one set of trials pepsin, the protease activator, cysteine, and the inhibitors  $\text{KBrCO}_3$  and Cebione, were added when the meal-water doughball was made. In another set these ingredients were added when the yeast was mixed in. The results obtained from predigesting the doughball four hours and then adding the other ingredients when the yeast was incorporated are given in Table XVII. It was necessary to make the doughball at first a little stiff and then add a small amount of water when the yeast was incorporated.

TABLE XVII

EFFECT OF PREDIGESTION AND THEN ADDING INHIBITORS, CYSTEINE AND ENZYMES AT THE TIME YEAST WAS ADDED

	Tenmarq		Blackhull	
	Meal	Flour	Meal	Flour
	<i>Min.</i>	<i>Min.</i>	<i>Min.</i>	<i>Min.</i>
Doughball (check)	125	90	61	108
Predigested doughball	172	80	107	103
Predigested doughball + 2 mg. $\text{KBrO}_3$	289	103	260	90
Predigested doughball + 2 mg. Cebione	225	82	171	101
Predigested doughball + 2 mg. cysteine	101	90	37	99
Predigested doughball + 2 mg. pepsin	82	99	49	110
Predigested doughball + 5 mg. diastase	166	93	105	114

It is evident that predigestion increased the time of the meal, but the effects on the flours were within experimental error. The addition of the inhibitors  $\text{KBrO}_3$  and Cebione as well as diastase further increased the time but cysteine and pepsin decreased the time. Hence, the changes in the physical properties of the dough due to predigestion increased rather than decreased the time.

The results of the trials in which the other ingredients were added at the same time the doughball was made are given in Table XVIII. These ingredients would then exert their influence during the time of the predigestion. The yeast was incorporated at the end of the four hour period.

TABLE XVIII

EFFECT OF PREDIGESTING WITH THE OTHER INGREDIENTS PRESENT

	Tenmarq		Blackhull	
	Meal	Flour	Meal	Flour
	<i>Min.</i>	<i>Min.</i>	<i>Min.</i>	<i>Min.</i>
Doughball (check)	131	99	56	105
Predigested with 2 mg. $\text{KBrO}_3$	275	106	193	81
Predigested with 2 mg. Cebione	255	103	213	112
Predigested with 2 mg. cysteine	142	83	64	96
Predigested with 2 mg. pepsin	85	73	43	97
Predigested with 5 mg. diastase	181	85	99	111

Whether these ingredients are added after or before the predigestion seems to have made very little difference on the time. Therefore it seems that the predigestion did not stimulate any enzymes in the meal or flour so as to affect the time. Since the inhibitors  $\text{KBrO}_3$  and Cebione were as effective in increasing the time whether added before or after the digestion period indicates that their chief influence is on the yeast.

### Effect of Pepsin on Flour and Meal

In the previous paper by Swanson and Dines (1939) it was shown that the pepsin decreased the time proportionately more on a long-time than on a short-time meal. Would there be the same proportionate effect on flours from wheats of longer and shorter time? The results obtained from long- and short-time wheats and the flours from the same are given in Table XIX. In each pair the short-time meal is placed first.

TABLE XIX  
EFFECTS OF PEPSIN ON FLOUR AND MEAL

Variety	Serial No.	No pepsin		With pepsin	
		Meal	Flour	Meal	Flour
		<i>Min.</i>	<i>Min.</i>	<i>Min.</i>	<i>Min.</i>
Turkey	23921	49	104	44	99
	23922	93	124	61	96
Kanred	23930	47	104	42	80
	23931	71	111	53	90
Tenmarq	23941	107	106	76	77
	23944	135		71	67
Blackhull	23950	68	104	59	85
	23951	87	98	57	71
Chiefkan	23958	52	87	78	46
	23961	86	125	66	74
Cheyenne	23967	109	120	75	96
	23970	134	125	121	105
Kawvale	23975	59	113	44	115
	23978	98	116	56	106

The figures in Table XIX show the following:

1. The time is longer on the flours than on the meals, but the differences between the flours and meals of long-time wheats are less than on short-time wheats. That is there is much less differentiation among varieties on the flours than on the meals.

2. When pepsin was used there was a reduction in time on both meals and flours and this was proportionately greater from long-time than from short-time wheats. The reduction on the flours was less than on the meals and hence pepsin made the differences less between the flours from long-time than from short-time wheats.

### Summary and Discussion

Tenmarq as a representative of long-time wheats and Blackhull and Chiefkan as representatives of short-time wheats were used for the

trials reported in this paper. The wheats were ground so as to have flour, bran, and shorts from each as well as meal.

Mixing the bran from a short-time wheat with the flour from a long-time wheat has the same effect as mixing the bran from a long-time wheat with the flour from a short-time wheat. Thus there does not seem to be any differences in the brans from the long- and the short-time wheats. The effects of heated bran were the same as the unheated and extracting with water did not change the effectiveness of bran in increasing the time on flour. Presoaking the bran was also without effect. Mixing bran with flour dilutes the gluten structure, but that such dilution does not necessarily decrease the time was shown by mixing starch with flour and with meal, since starch had little effect on the time of the flours and on the meal from Chiefkan. On Tenmarq meal the time was reduced with the increasing amounts of starch and when the starch was a little over half the mixture, the time differences on Tenmarq and Chiefkan disappeared.

Commercial wheat germ shortened the time on both the flours and the meals. The same was also true of the water extract of the germ. Heating the germ destroyed the potency of the water extract. Germinating the wheat made the time shorter. Merely soaking at 60°C. and 27°C. also made the time shorter. Incorporating meal from germinated wheat made the time shorter. That the effects of germinated meal were not due to an increase in diastase was shown by the non-effect obtained from adding the commercial malted wheat flour to meals and to flours. Commercial malted wheat flour increased the time on Tenmarq meal; hence whatever protease may be present had no effect.

Increasing the amount of yeast decreased the time but increasing the amount of sugar lengthened it. However, even with varying amounts of sugar and yeast the time differences between the Tenmarq and the Blackhull meals persisted.

Varying moisture and temperature conditions during storage have little effect on time unless they are extreme.

Cebione and  $\text{KBrO}_3$  increased the time, but pepsin and wheat germ may overcome their effects. Predigesting the meal, which causes important physical changes in the dough, has but little effect on time. Pepsin will reduce the time proportionally more on long-time meals and flours than on short-time meals and flours. Hence the main effect of pepsin is to obliterate differences which are obtained on the wheat meals from different wheat varieties.

Thus while various substances may be used either to increase or shorten the time the exact reason for the long time on some wheat meals and the short time on others is not entirely clear. The bran

itself does not seem to cause the differences. While proteases including those present in the wheat germ decrease the time, their ultimate effect is to remove differences between the long- and the short-time wheats. There seems to be some inherent factor in the gluten which causes the differences in time since they will persist under a number of manipulations, although they can be obliterated. But why the same time differences are not obtained on the flours as on meals needs further investigation.

Since the wheat-meal-time-fermentation test may be influenced by so many factors it must be used with caution in evaluating the quality of wheat varieties. Much more needs to be known about the factors which influence the breaking of the doughball at the dough-water interface. It is also evident from the data obtained in this series of papers that the problem must be attacked from several angles.

#### Literature Cited

Swanson, C. O.

1937 Factors which influence the results in the wheat-meal-time-fermentation test. *Cereal Chem.* **14**: 419-433.

1939 The wheat-meal-time-fermentation test. III. Effect of bran, proteases, and activators on the "time" of flour. *Cereal Chem.* **16**: 365-376.

— and Dines, F. T.

1939 The wheat-meal-time-fermentation test. II. Effect of proteases, protease activators, and protease inhibitors. *Cereal Chem.* **16**: 168-177.

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## STUDIES ON WHEAT STARCH. IV. FRACTIONATION AND AMYLASE HYDROLYSIS <sup>1</sup>

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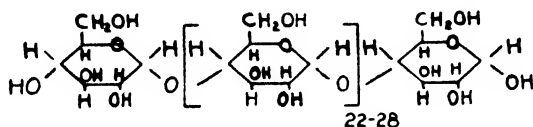
(Read at the annual meeting, May 1939)

When a starch solution is hydrolyzed by beta-amylase the hydrolysis will proceed rapidly until about 50% to 60% is converted into maltose. The remaining part of the starch is relatively resistant to further action by beta-amylase. This portion of the starch which remains after beta-amylase hydrolysis is generally referred to as erythrogranulose, using Wijsman's (1889) terminology. Other names used for this fraction are alpha-amylodextrin (Baker, 1902), alpha-starch (Klinkenberg, 1932), and beta-dextrin (Myrbäck, 1937). While erythrogranulose is the name most commonly employed, it is really a misnomer since the material gives a blue color with iodine.

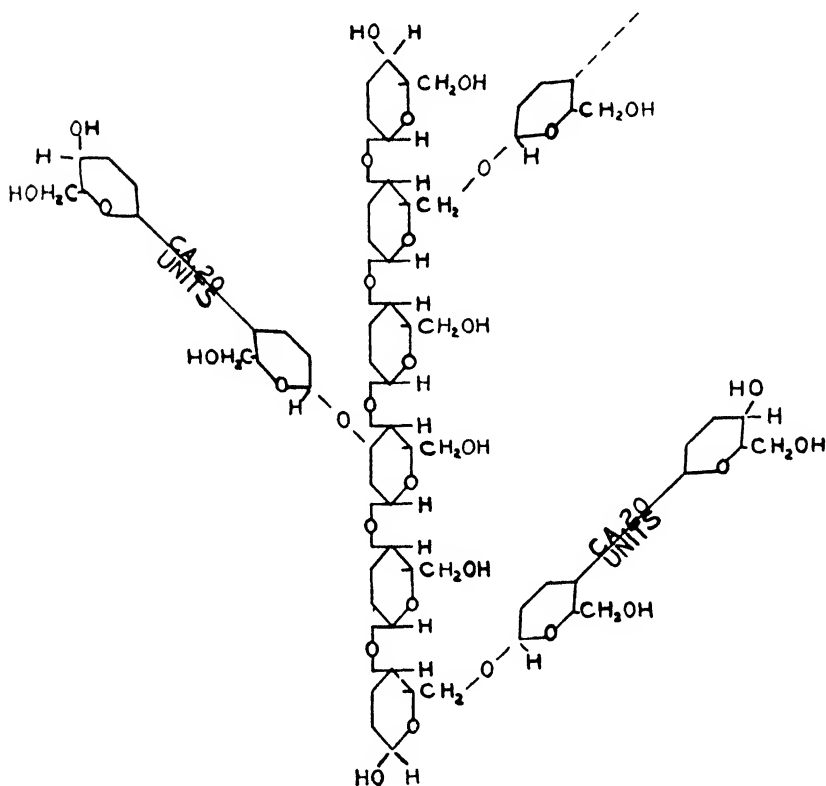
Myrbäck (1937) reported his belief that beta-amylase attacks only the straight chain part of the starch molecule which corresponds to the Haworth model, and that the incomplete hydrolysis of starch by beta-

<sup>1</sup> Paper No. 1748, Journal Series, Minnesota Agricultural Experiment Station.

amylase is due to certain anomalies in the starch molecule, such as phosphorus in ester linkages, or branched chains in the molecule, as suggested by Staudinger and Husemann (1937). The Haworth straight chain model and the Staudinger-Husemann branched chain model are reproduced in Figure 1.



### HAWORTH MODEL



### STAUDINGER-HUSEMANN MODEL

Fig. 1. Showing the Haworth straight chain model and the Staudinger-Husemann branched chain model for the molecular structure of starch.

In this investigation wheat starch was separated into various fractions which were then studied as to their susceptibility to amylase action.



### Experimental

Commercial wheat starch (I) was pulverized in a rod mill and quantitatively separated into amylopectin (II) and amylose (III) by the electrodecantation method described by Stamberg and Bailey (1939a). A part of the same pulverized wheat starch was then hydrolyzed by purified beta-amylase. Several portions of the enzyme were added until no further action was observed as determined by maltose tests. The remaining erythrogranulose was precipitated by addition of ethyl alcohol to a 65% concentration. The white precipitate (IV) was recovered by centrifuging and washed several times with 65% alcohol to remove the maltose. About 85% of the theoretical yield of the erythrogranulose, as based on the amount of the starch not hydrolyzed by beta-amylase, was thus recovered. The erythrogranulose (IV) was practically non-reducing and gave a blue color with iodine. It was also fractionated into amylopectin (V) and amylose (VI) by the electrodecantation method in exactly the same manner as the pulverized wheat starch.

Phosphorus determinations were made on the starch and the fractions by the technique referred to by Stamberg and Bailey (1939a). The results obtained from duplicate experiments are shown in Table I, which includes the percentage of each fraction and the phosphorus content.

TABLE I  
WHEAT-STARCH FRACTIONATION RESULTS AND  
PHOSPHORUS CONTENT OF THE FRACTIONS

Starch fractions	Fractionation results			Phosphorus content		
	1	2	Av.	1	2	Av.
I. Starch	%	%	%	%	%	%
II. Amylopectin from I	21.5	21.8	21.65	0.066	0.065	0.066
III. Amylose from I (by difference)	78.5	78.2	78.35	0.233	0.256	0.244
IV. Erythrogranulose from I after beta-amylase action	50.1	49.7	49.9	0.004	0.005	0.005
V. Amylopectin from IV	50.1	49.7	49.9	0.073	0.075	0.074
VI. Amylose from IV	27.3	26.2	26.75	0.293	0.284	0.289
	72.7	73.8	73.25	0.004	0.002	0.003

Alpha- and beta-amylases were prepared by the technique described by Stamberg and Bailey (1939b). The action of the amylases at 30° on the various starch fractions was studied by using 25 cc. of 1% substrate buffered to pH 5.1 with a citrate buffer, and 12.5 mg. of the enzyme. The rate of hydrolysis was followed by maltose determina-

tions using the ferricyanide method. The results of these tests are shown graphically in Figure 2. Beta-amylase was allowed to act alone for 12 hours, at which time the action was apparently complete as indicated by the curves approaching an asymptote. Alpha-amylase was then added and the hydrolysis continued for another 12 hours.

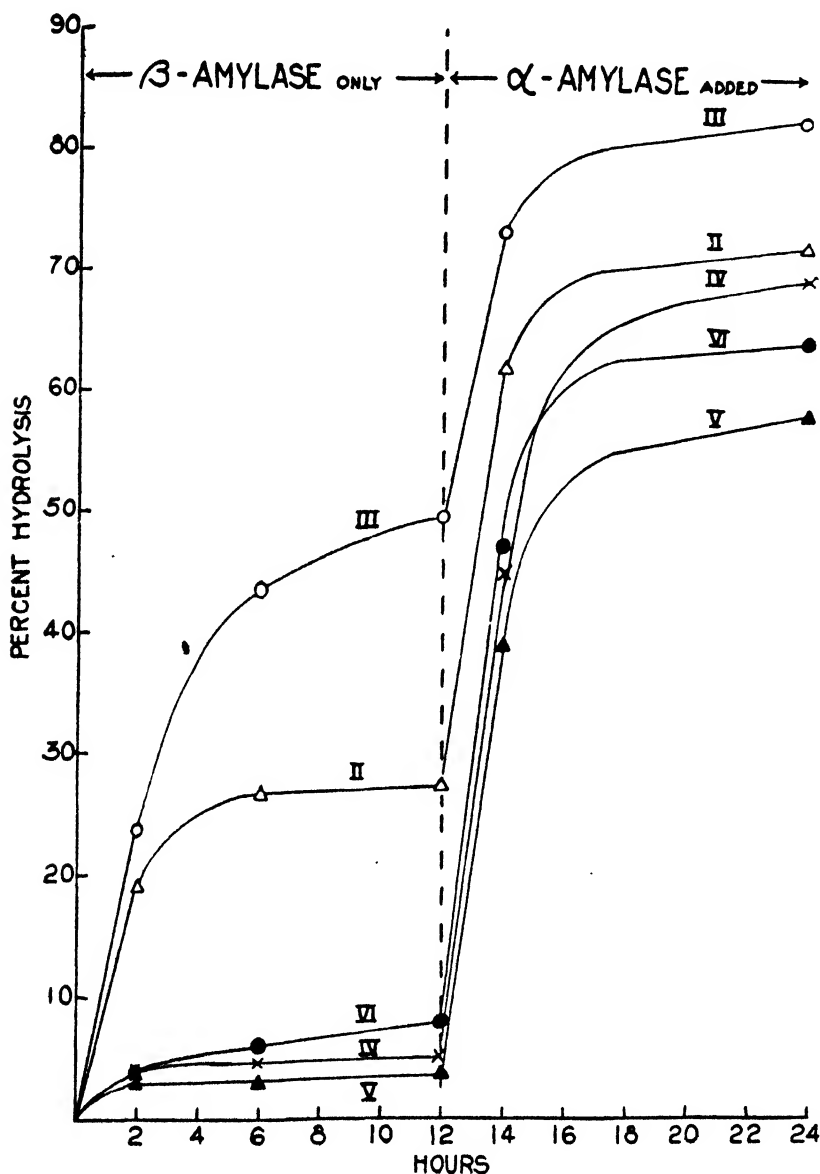


Fig. 2. Results of enzyme hydrolysis. Beta-amylase acted alone for 12 hours, after which alpha-amylase was added. The substrates were amylopectin (II) and amylose (III) from starch, and amylopectin (V) and amylose (VI) from erythrogranulose (IV).

### Discussion

To all visual appearances the amylopectin and amylose fractions from the erythrogranulose were much like those from the starch in that the amylopectin preparations were turbid, viscous, and gave a red-violet iodine color, while the amylose preparations were clear, fluid, and gave a brilliant blue color with the same amount of iodine. The data in Table I show that the respective amylopectin fractions contain practically all of the phosphorus of the starch, or the erythrogranulose respectively.

It is interesting to note that the phosphorus content of the erythrogranulose is only slightly higher than that of the starch and quite in relation to the higher amylopectin content of the erythrogranulose. This suggests that the portion of the starch hydrolyzed by beta-amylase contained phosphorus and that beta-amylase action is not inhibited by the presence of phosphorus in the starch.

Beta-amylase hydrolyzed the erythrogranulose (IV) and its fractions (V) and (VI) only slightly, but attacked the amylopectin (II) and the amylose (III) from the starch quite readily. The rapid activity which followed upon the addition of alpha-amylase indicates that this enzyme readily hydrolyzes erythrogranulose and also the amylopectin and amylose derived from this fraction.

Myrbäck (1937) suggested that beta-amylase action is blocked by the branched chains of the carbohydrate units of the starch or by fatty acids or phosphorus in ester linkages. In this study the phosphorus-free amylose (VI) from the erythrogranulose was resistant to beta-amylase action and the amylopectin (II) from the starch which had a high phosphorus content was quite readily hydrolyzed. It is unlikely that fatty acids were present in the phosphorus-free amylose (VI) to block the beta-amylase action, since the fatty acids are usually found in the amylopectin fraction according to Taylor and Werntz (1927). The recent work by Schoch (1938, 1939) indicates that fatty acids in starch are not chemically combined and can be removed by extraction with various fat solvents. The samples handled in these experiments were entirely too small for fatty acid determinations.

The data indicate that the resistance of erythrogranulose to beta-amylase action is due primarily to a difference in structure of the carbohydrate component of the starch remaining after beta-amylase action as compared to the fraction hydrolyzed by beta-amylase. This may be due to branched chains in the molecular structure of the starch as shown by the Staudinger-Husemann model. Branched systems of the carbohydrate units appear further supported by the fact that Myrbäck

(1938a, 1938b) has isolated a trisaccharide from starch which probably had a linkage other than that occurring in maltose.

If the blocking of beta-amylase action is due to a branched chain structure of the starch molecules the question remains whether that part of the starch which is hydrolyzed by beta-amylase is made up entirely of straight chain molecules of the Haworth type, or whether in the branch chain molecules the extended parts of the branches are readily hydrolyzed by beta-amylase until a certain proximity to the branching units is reached. The latter theory with all molecules having the same type of structure appears more likely.

The phosphorus-free amylose fraction (VI) obtained by electrodecantation from the erythrogranulose constitutes a fraction with a high concentration of the probable linkages in the starch molecules that account for the blocking of beta-amylase activity, and also contains the linkages split by alpha-amylase.

### Summary

Wheat starch (I) was separated into amylopectin (II) and amylose (III) by electrodecantation. After hydrolysis of wheat starch by beta-amylase the residual erythrogranulose (IV) was likewise fractionated into amylopectin (V) and amylose (VI). The erythrogranulose contained 26.75% amylopectin (V) and the starch (I) contained 21.65% amylopectin (II) with the technique used.

The amylopectin fractions (II and V) contained practically all of the phosphorus with only a trace present in the amylose fractions (III and VI). In appearance and iodine color the two amylopectin fractions were alike and the two amylose preparations were also similar in these respects.

Beta-amylase hydrolyzed the amylopectin (II) and the amylose (III) from starch to an appreciable extent, but this enzyme had very little action on the amylopectin (V) and amylose (VI) from the erythrogranulose (IV).

It appeared evident that beta-amylase action is not blocked, although perhaps retarded, by the phosphorus in the starch, and that the blocking of beta-amylase action is apparently due to the structure of the carbohydrate in the residual erythrogranulose of starch.

### Literature Cited

- Baker, J. L.  
1902 The action of ungerminated barley on starch. *J. Chem. Soc.* **81**: 1177-1185.  
Klinkenberg, G. H.  
1932 Über die Spezifität der Amylasen. *Z. Physiol. Chem.* **212**: 173-195.  
Myrbäck, K.  
1937 Über den Enzymatischen Abbau der Stärke. *Skand. Arch. Physiol.* **77**: 58-59.

- 1938a Über Grenzdextrine und Stärke. *Biochem. Z.* **297**: 179-183.  
1938b The constitution of dextrin and starch. III. Trisaccharide from starch. *Svensk. Kem. Tid.* **50**: 27-31. Through *C. A.* **32**: 3652.  
Schoch, T. J.  
1938 Absence of combined fatty acids in cereal starches. *J. Am. Chem. Soc.* **60**: 2824.  
1939 Fatty acids in cereal starches. Abstract of 97th Meeting Am. Chem. Soc., Div. Sugar Chem., p. 8, April 3-7.  
Stamberg, O. E., and Bailey, C. H.  
1939a Studies on wheat starch. I. The amylopectin and amylose content of various wheat starches. *Cereal Chem.* **16**: 309-319.  
1939b Studies on wheat starch. II. The action of amylases on raw wheat starches. *Cereal Chem.* **16**: 319-330.  
1939c Studies on wheat starch. III. The action of amylases on wheat amylopectin and amylose. *Cereal Chem.* **16**: 331-335.  
Staudinger, H. and Husemann, E.  
1937 Über Konstitution der Stärke. *Ann.* **527**: 195-236.  
Taylor, T. C. and Werntz, J. H.  
1927 Properties of corn starch. *J. Am. Chem. Soc.* **49**: 1584-1588.  
Wijsman, H. P.  
1889 Through G. H. Klinkenberg. Über die Spezifität der Amylasen. *Z. Physiol. Chem.* **209**: 253-268 (1932).

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## PROTEIN FILMS AND THE SUSCEPTIBILITY OF RAW STARCH TO DIASTATIC ATTACK <sup>1</sup>

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(Received for publication November 6, 1939)

It is generally recognized that raw-starch preparations of different botanical origin may vary greatly in the ease with which the granules are attacked by diastatic enzymes. It is also rather generally recognized that the raw granules in different samples of wheat flours may show different susceptibilities toward diastatic attack (*e.g.*, Andrews and Bailey, 1934; Malloch, 1929; Mangels 1926, 1936; Rumsey 1922; Stamberg and Bailey, 1939), and that this property may be one factor involved in the variable gassing power exhibited by different flours.

Both Alsberg (1928) and Kemp (1936) have suggested the possibility that the starch granules in wheat flour may be coated with protein films, although neither of these authors has suggested exactly the viewpoint which we propose. Alsberg speaks of the effect on the resistance of starch to diastatic attack as possibly due to the starch being locked up in a "gluten matrix" through which "colloidal membrane" the attacking diastase must diffuse before it can attack the granule. Kemp speaks of "flour particles" which, under the conditions of his experimental technique, may mean starch or, as well, fragments of the

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<sup>1</sup> Paper No. 1750 Journal Series, Minnesota Agricultural Experiment Station.

wheat-berry endosperm, and from electrokinetic studies he concludes that such "flour particles" have about 80% of their surface covered with gluten. It should be noted that he bases this conclusion on calculations involving studies of the cataphoretic behavior of both wheat starch and gluten particles. No suggestion is made that the starch studied might have a portion of its surface protein-covered. If such were the case, his calculations would be invalidated.

Colloid chemists have recently made use of the property of proteins to coat inert surfaces with an adherent protein film in order to study the cataphoretic behavior of *soluble* proteins. Such coated particles behave electrokinetically as if they were solid masses of protein and migrate as such in an electric field. Loeb (1922-3, 1923-4 a, b, c) in this manner studied the electrokinetic behavior of gelatin and egg albumin coated upon collodion droplets and Abramson (1928) and Abramson and Freundlich (1928) later used gelatin, egg albumin, and hemoglobin coated upon zinc dust, quartz, and glass. Many recent studies on the physicochemical properties of proteins have been concerned with various proteins adsorbed as films on quartz, glass, or carbon particles. Thus from these and many other types of investigations dealing with protein films, it has become axiomatic that inert surfaces in contact with most protein solutions become coated with an adsorbed protein film, and that such systems behave physicochemically as though they were wholly protein.

Since, in the process of growth, the starch granules in the wheat berry are laid down simultaneously with the protein, or in any event in a protein-containing environment, it seemed probable that such granules are more or less completely incased in a protein film and possibly it is the variable completeness or incompleteness of this film or its varied thickness that determines the variable resistance of raw starch to diastatic attack. From all available evidence, and particularly from electrokinetic data, it should be impossible to remove such films completely by any simple washing processes. There is no direct evidence that the nitrogen content of raw starch is directly correlated with its diastatic susceptibility, but this is not necessarily an argument against the film hypothesis since a variable amount of the nitrogen-containing material may not be exposed in surface films but may be buried in the interior of the starch granule.

### Experimental

In order to test the film hypothesis we have studied the actions of takadiastase upon raw wheat starch under such conditions as to insure the presence of more or less complete protein films surrounding the granules.

The starch was a sample prepared in this laboratory by Dr. Henry Rees from Thatcher wheat. The method of preparation was such as to remove practically all broken or small granules, leaving a final product of remarkably uniform granule size. The final product was air-dried and no heating or chemical treatments were resorted to at any stage in the starch-preparation procedure.

The takadiastase used was a highly active product in the form of a white powder, prepared for us by the research laboratories of the Parke-Davis company. It had many times the activity of the usual commercial preparations.

The proteins used were gelatin, gliadin, and egg albumin. The gelatin was derived from a commercial sample which had been purified by extensive electrodialysis and dried after becoming isoelectric (pH 4.70). The ash content was 0.13% but this relatively high ash content was due to contamination with inert porcelain dust from ball-mill grinding. The gliadin was a sample isolated by the Blish and Sandstedt acetic acid method and used for previous studies from this laboratory (Sinclair and Gortner, 1933). The egg albumin was prepared by the Sorensen method, recrystallized twice, and electrodialyzed until isoelectric and free from sulfates. It was kept in solution under toluene as a preservative.

The plan of the experiments was as follows: The raw starch (1 g. dry weight) was digested with 10 mg. of takadiastase in an acetic acid-sodium acetate buffer system in a final solution volume of 46 cc. at pH 4.80-4.88 and 38°C. for a 3-hour period and the reducing sugars so formed determined by the Blish and Sandstedt (1933) method. The values so obtained formed the "check" series.

An identical series was run simultaneously in the presence of varying amounts of protein—the protein present ranging from 0.001% to 0.250% of the final volume concentration.

In a third series the raw starch, suspended in 50 cc. of protein solution ranging from 0.001% to 0.250% concentration, was allowed to remain in these solutions for 2-3 hours with frequent shaking in order to allow maximum film formation to take place. The starch was then sedimented by centrifuging, the supernatant liquid was decanted, and the starch precipitate washed once in the centrifuge with an additional 50 cc. of water and the starch precipitate then digested as in the other experiments.

In a fourth series the experiments were conducted exactly as in series III excepting that 1 mg. of activated papain was added to the diastase digest to possibly break down adherent protein films which might be present.

The results of these experiments are shown in Table I where the percentage increase (+) or decrease (−) in reducing sugars formed over the amount characteristic of the "check" samples is reported.

TABLE I

PERCENTAGE INCREASE (+) OR DECREASE (−) IN MALTOSE PRODUCTION WHEN RAW WHEAT STARCH WAS DIGESTED WITH TAKADIASTASE IN THE PRESENCE OF ADDED PROTEINS

Series II, protein added to digest and remaining in digest during period of diastase action. Series III, protein added to starch, later starch washed to remove all except adherent protein films and then digested with diastase. Series IV, identical with Series III except that 1 mg. of activated papain was added to digest to possibly attack protein films.

Protein concn. %	Series II %	Series III %	Series IV %
GELATIN EXPERIMENTS			
0.001	−12.6	− 3.5	− 7.3
0.005	+ 1.0	−11.0	− 5.2
0.010	0.0	−20.3	−21.6
0.025	−18.5	−46.3	−26.3
0.050	+ 1.0	−55.5	−41.0
0.100	−26.2	−48.0	−46.0
0.250	−81.6	−44.0	−53.9
GLIADIN EXPERIMENTS			
0.001	+12.8	+ 0.5	+17.3
0.005	+ 8.2	+12.4	+ 6.0
0.010	+14.9	−53.6	+ 8.1
0.025	− 2.1	−56.2	+15.1
0.050	− 4.1	−49.0	− 6.5
0.100	+11.3	−56.7	− 1.6
0.250	+ 5.6	−69.6	0.0
EGG-ALBUMIN EXPERIMENTS			
0.001	+ 1.2	+ 2.5	− 3.1
0.005	−11.2	+ 8.6	− 2.7
0.010	− 7.9	−35.9	− 6.2
0.025	−40.7	−31.0	− 6.9
0.050	−34.0	−33.5	−12.0
0.100	−52.7	−24.5	−34.3
0.250	−71.8	−30.6	−37.3

### Discussion

While the data show some inexplicable variations, certain observations are clear. From all available electrokinetic literature there seems to be no question but that gelatin and egg albumin in nearly all concentrations of protein used in the present studies would cause the formation of protein films on any surfaces exposed to such solutions. We believe that such films were formed or added to the starch surfaces



in our experiments and that such films were responsible for reduction of attack by diastase. The difference between series II and series III in both the gelatin and gliadin experiments is surprisingly in favor of the washed film-covered starch in contrast to the systems when the major portion of the protein remained in the supernatant liquid. Perhaps the protein in the supernatant liquid acted as an "enzyme protector" for the diastase, for all enzyme action as measured by end products is the net result of rate of enzyme attack of substrate and rate of enzyme inactivation due to environmental conditions, and proteins have been shown in many studies to retard the rate of enzyme inactivation. However, whatever the effect of excess protein may be, we would lay particular emphasis on the striking reduction in maltose production in all three experiments of series III where the protein present is probably largely in the form of protein films. Those data indicate strongly that such adherent protein films may have much to do with the problems of diverse starch susceptibility to diastatic attack.

The addition of papain in the gliadin experiments, series IV, apparently largely removed the inhibiting films. Papain also apparently showed some effect in the egg albumin experiments, especially when the films were formed in the lower protein concentrations and there is a more doubtful effect of papain shown in the gelatin experiments. The somewhat anomalous papain effect in the different experiments may be coupled with (1) different rates of attack of papain on the different protein films, (2) diastase digestion and hence inactivation, and (3) papain, or impurities present in the enzyme preparation may in turn form adherent films on the starch granules. More work must be done before all of the various factors which may be involved are separated and quantitatively evaluated.

### Summary

The hypothesis is presented that the variable susceptibility of raw starch to attack by diastatic enzymes may be due, at least in part, to the starch granules being more or less completely incased by a protein film. A starch preparation composed almost wholly of uniform-sized unbroken granules was so treated as to induce film formation in solutions of gelatin, gliadin, and egg albumin. Marked reduction in the ease with which such treated starch was attacked by takadiastase was noted in most experiments. Certain anomalies were observed which require further study.

## Literature Cited

- Abramson, H. A.  
1928 A new method for the study of cataphoretic protein mobility. *J. Amer. Chem. Soc.* **50**: 390-393.
- and Freundlich, H.  
1928 Über die kataphoretische Wanderungsgeschwindigkeit größer Teilchen in Solen und Gelen. I. *Z. physikal. Chem.* **128**: 25-38. II, *ibid.* **133**: 51-68.
- Alsberg, C. L.  
1928 The role of starch in breadmaking, in P. R. Walton, A comprehensive survey of starch chemistry, Vol. I, pp. 87-99, Chemical Catalog Co., N. Y.
- Andrews, J. S., and Bailey, C. H.  
1934 The amylases of normal and germinated wheat flours. *Cereal Chem.* **11**: 551-556.
- Blish, M. J., and Sandstedt, R. M.  
1933 An improved method for the estimation of flour diastatic value. *Cereal Chem.* **10**: 189-202.
- Kemp, I.  
1936 The surface analysis of particles of certain wheat flours. *Trans. Faraday Soc.* **32**: 837-843.
- Loeb, Jacques  
1922-3 The influence of electrolytes on the cataphoretic charge of colloidal particles and the stability of their suspensions. II. Experiments with particles of gelatin, casein and denatured egg albumin. *J. Gen. Physiol.* **5**: 395-413.  
1923-4 On the location of the forces which determine the electrical double layer between colloidal particles and water. *Ibid.* **6**: 105-129.  
1923-4 The influence of the chemical nature of solid particles on their cataphoretic P.D. in aqueous solutions. *Ibid.* **6**: 215-237.  
1923-4 Hydrophilic and hydrophobic colloids and the influence of electrolytes on membrane potentials and cataphoretic potentials. *Ibid.* **6**: 307-328.
- Malloch, J. C.  
1929 Studies on the resistance of starch to diastatic action. *Can. J. Research* **1**: 111-147.
- Mangels, C. E.  
1926 Factors affecting the diastatic activity of wheat flour. *Cereal Chem.* **3**: 316-322.  
1936 Varietal and regional variation in durum wheat starches. *Ibid.* **13**: 221-233.
- Rumsey, L. A.  
1922 The diastatic enzymes of wheat flour and their relation to flour strength. *Am. Inst. Baking, Bul.* **8**.
- Sinclair, W. B., and Gortner, R. A.  
1933 Physico-chemical studies on proteins. VII. The peptization of gliadin by solutions of inorganic salts. *Cereal Chem.* **10**: 171-188.
- Stamberg, O. E., and Bailey, C. H.  
1939 Studies on wheat starch. II. The action of amylases on raw wheat starches. *Cereal Chem.* **16**: 319-330.

# STUDIES OF THE USEFULNESS OF A MOTOR-DRIVEN SHEETER IN TEST BAKING <sup>1</sup>

PAUL P. MERRITT,<sup>2</sup> MAX C MARKLEY,<sup>3</sup> and ERNST ROTHHOLZ <sup>3</sup>

(Read at the Annual Meeting, May 1939)

The introduction of a low-cost power-driven sheeting roll for small loaf test baking, by the National Mfg. Co. of Lincoln, Nebraska, offers a possible solution to the problem of mechanizing certain phases of the test baking procedure. The evaluation of such an instrument can only be made from a combination of critical tests and from day-to-day observations of it when placed in actual use in the test bakery.

This sheeter is designed for two-pass operation with first-pass settings from 3/16" to 5/16" by 1/32" steps and second-pass settings from 1/8" to 1/4". The optimum settings for each pass were determined by a series of baking tests employing most of the practical combi-

TABLE I  
EFFECT OF VARYING THE SHEETING ROLL SETTING  
UPON THE VOLUME OF THE BREAD

First pass	Second pass	Loaf volume
<i>In.</i>	<i>In.</i>	<i>ML.</i>
Hand Molded	—	600
3/16	1/8	570
3/16	5/32	575
3/16	3/16	560
7/32	1/8	575
7/32	5/32	580
7/32	3/16	590
7/32	7/32	580
1/4	1/8	535
1/4	5/32	560
1/4	3/16	600
1/4	7/32	595
1/4	1/4	555
9/32	1/8	560
9/32	5/32	575
9/32	3/16	600
9/32	7/32	605
9/32	1/4	565
5/16	7/32	595
5/16	1/4	580
Hand Molded	—	590

nations. The loaf-volume data from this series, in which a medium-strength flour was employed, are given in Table I. For this flour it appeared that a first pass of 1/4" to 9/32" and a second pass of 3/16" to 7/32" gave the best results. However, it was later found that a first-

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pass setting of  $1/4''$  was too tight for very elastic or bucky doughs, and also that a second-pass setting of  $7/32''$  would not remove all gas from such doughs, so a first-pass setting of  $9/32''$  and a second-pass setting of  $3/16''$  was selected as a working standard. This combination has been found in practice to work well with flours of all types, though possibly not quite optimum for some extreme types.

For a more critical examination of the motor-driven sheeter a series of baking tests involving both hand and machine punching and molding and three operators was undertaken. In this investigation three flours of quite widely varying dough characteristics were chosen. One was a flour milled from Minnesota winter wheat which yielded doughs that were very soft and pliable. The second flour was experimentally milled from very strong Montana spring wheat and was extremely elastic or bucky. This flour was intentionally undermixed in order to exaggerate its normal bucky nature. The third flour was a composite of Kansas bakery type flours which yielded a well mellowed dough at ordinary fermentation times. The loaf volumes from these particular flours as baked were not exactly in line with their strength ratings.

Doughs were made from 400 g. of flour using the basic formula with the exception of double sugar. The first two flours were mixed in the McDuffee-Hobart mixer and the Kansas flour in the Fleischmann mixer. Nine doughs were mixed from each flour. From the mixer each dough was divided into four 160-g. aliquots and fermented and baked according to the basic schedule. Two operators worked simultaneously, each punching and molding one aliquot by hand and the other by machine. The punching was done by passing the dough once through the sheeter at  $9/32''$ . The sheeting for molding was done by two passes of  $9/32''$  and  $3/16''$  each. The operators varied in experience. Operators *A* and *B* were long experienced with the small-loaf technique, while operator *C* was relatively inexperienced. Operator *A* had been using the sheeter for several months while *B* and *C* had used it but little prior to these tests.

The resulting loaves were measured for volume and scored for type, crust color, crumb color, texture, and grain. The color of either crust or crumb did not appear to be altered by either treatments or operators. Loaf type, grain, and texture tended to be more uniform for the machine-handled loaves than for the hand ones. The greatest effect was upon loaf volume.

The machine-handled loaves of both the Minnesota winter and the Montana spring flours were significantly higher in volume for both operators *A* and *B* than the hand-treated loaves, as can be seen in Table II. The Kansas flour behaved in a different manner, giving

essentially the same volume by both methods for operator *B* and slightly lower volume by machine for operator *C*. The variability of the individual operator was reduced by the introduction of the machine. The average standard deviation for the hand-treated loaves was 19.5 cc. and for the machine-handled ones 16.5 cc. When the increase in volume is taken into account the actual reduction in variability is a little greater.

TABLE II  
COMPARISON OF HAND AND MACHINE SHEETING OF DOUGHS

	Operator A		B		A and B		A and B
	Hand	Ma- chine	Hand	Ma- chine	Hand	Ma- chine	Hand and Machine
MINNESOTA WINTER WHEAT FLOUR							
Mean	665.8	706.7	647.1	702.5	656.5	704.6	680.5
Standard deviation	15.7	18.7	22.9	22.1	20.2	20.6	15.5
Coefficient of variation	2.4	2.7	3.5	3.2	3.1	2.9	2.3
Standard error of mean	4.8	5.6	6.9	6.7	6.1	6.2	4.7
MONTANA SPRING WHEAT FLOUR							
	Operator A		B		A and B		A and B
	Hand	Ma- chine	Hand	Ma- chine	Hand	Ma- chine	Hand and Machine
Mean	712.8	734.6	708.2	751.9	710.5	743.2	726.8
Standard deviation	17.8	15.8	18.7	14.8	19.6	18.9	12.8
Coefficient of variation	2.5	2.2	2.6	2.0	2.8	2.5	1.8
Standard error of mean	5.6	5.0	5.9	4.7	6.2	6.0	4.0
KANSAS HARD WINTER WHEAT FLOUR							
	Operator B		C		B and C		
	Hand	Machine	Hand	Machine	Hand	Machine	Hand and Machine
Mean	621.5	623.0	604.0	582.0	607.6		
Standard deviation	16.0	11.0	21.8	16.6	12.0		
Coefficient of variation	2.6	1.8	3.6	2.9	2.0		
Standard error of mean	5.3	3.7	7.3	5.5	4.0		

In this study the inter-operator variability was not reduced by the sheeter. However, the operators were not a random selection since both *A* and *B* had had closely parallel experience in baking small loaves, and had learned to mold these small doughs from the same technician. Operator *C* was trained in small loaf technique by both *A* and *B*. Other observations have indicated that with operators varying widely in molding technique there is a reduction in inter-operator variability with the sheeter.

In addition to the reduction in variability the introduction of the sheeter reduces the time required to mold the loaves. This time-saving becomes important when long lines of bread are being baked on short-interval schedules and for variable fermentation studies. It enables operations to be carried out more nearly on the scheduled time.

The sheeter has another advantage. This is the lessening of operator fatigue. In the baking of lines of 30 or more doughs operator fatigue becomes an important factor, causing a greater variability in the later doughs than in the earlier ones. The operator fatigue was not accounted for in the experiments here since only 9 doughs were hand molded at 10-minute intervals.

### Conclusions

The motor-driven sheeting rolls tend to reduce the variability in replicate bakes by individual operators, but will not account for all of the variability encountered.

The sheeter fits well into baking schedules because of its saving in molding time.

The reduction in operator fatigue is an important advantage of mechanical dough sheeting over hand operation.

In the opinion of the authors the motor-driven dough sheeter, fitted for at least two settings of the rolls, is an important piece of baking equipment and should be specified as official.

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## TEST BAKING PAN DESIGN<sup>1</sup>

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(Read at the Annual Meeting, May 1939)

The matter of correct dimensions for the pans to be used with the small-loaf baking test is one on which there are nearly as many opinions as there are test bakers. At the present time two alternative pans are specified which are representative of the two extremes in design. Neither of these pans has the 100-mm. inside bottom length which was adopted at the 1938 convention as a standard length for the baking pan, so it became imperative to redesign the standard pans. Neither of the present pans was designed to fit the present-day baking test.

The high-form pan, as specified by Blish (1928), was developed by

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<sup>1</sup> Subcommittee report, 1938-39 Committee on Standardization of Laboratory Baking.

the late E. E. Werner as a reduced model of the old Howard expansion loaf pan. The basic test, as devised by Dr. Werner, was a test of the diastatic activity or gassing power of a flour rather than the strength. For this purpose the high-form pan is very well suited. However, with the development of physical and chemical tests for the determination of diastatic activity in recent years there is now less emphasis placed upon the diastatic activity in the baking test and more emphasis is being laid upon the factors involved in the determination of the relative strengths of flours. When the workers in the Canadian plant-breeding laboratories tried out the basic test for the evaluation of their strong spring wheats it was soon found that the high-form pan did not give the differentiation in loaf volume which was to have been expected from the relative protein contents of wheats of similar genetic constitution, so they tried a low-form pan and found that it would more sharply differentiate the loaf volumes of flours milled from wheats of high protein content. This low-form pan was a baker's sample pan which had been tried on a limited scale by Herman and Hart (1927).

However, the low-form pan was found to have certain defects. It is too small in volume to yield well-shaped loaves of 600 cc. or greater volume. With the resulting tendency to the production of balloon tops there is less differentiation in loaf-type scores between flours than with the higher pan. Also the grain is not as uniform as in the high-form pan because of the tendency for large holes to form in a layer just below the upper crust. The grain in the low-form pan is usually spherical while in the high pan it is often vertically elongated. This elongated grain structure has long been considered the ideal type in laboratory bread scoring, but the work of Moen (1935) has cast a serious doubt upon this assumption. He found that there was little correlation between the grain of loaves baked in the high-form pans and that of loaves baked under commercial formulas and with standard commercial pans. So it appears better to use a pan which tends to produce a spherical grain rather than one which will give a high percentage of loaves with the elongated type of structure. Several attempts have been made to design pans which will be better than either of the two types. The Pioneer Section has developed a pan which has met with favor in that region. Miller and Whiteside (1938) have made a large low-form pan for use with loaves of 800 cc. or larger.

The Pioneer pan was shown by Davis, Leatherock, and Putnam (1936) to be superior to either of the two specified types for loaves of approximately 600-cc. volume. This pan is similar to the low-form pan with the exception of greater bottom area. It appears to be well adapted to differentiating flours in the low and medium volume ranges,

but is too small to be satisfactory for the larger loaves. Also the length of the bottom is greater than 100 mm., which has been adopted as standard. The Miller and Whiteside pan is very satisfactory for high-protein flours but does not produce satisfactory loaves of low volume.

After considering all these factors it appeared inadvisable merely to make a change in the bottom length of either or both of the standard pans. The only practical recourse seemed to be to design a completely different pan which would combine the advantages of all types with none of the disadvantages. Of course it is improbable that this ideal could be attained, but it appears to be a goal worthy of some effort. The length of the pan has been previously fixed at 100 mm. The width of the bottom has been shown to have definite limits. The height should not be less than that of the low-form pan and not more than that of the high form. The range of dimensions is thus limited to a comparatively narrow selection. After some preliminary trials a pan having a bottom of  $100 \times 60$  mm., top of  $115 \times 75$  mm. and 60-mm. vertical depth was constructed and subjected to extended tests in several laboratories. This pan will be referred to as the intermediate type since it is intermediate between the extremes of the four types of small-loaf pans in every dimension.

In the author's laboratory special attention was given to loaf type and to grain. Wheats of all types common in the world wheat trade were baked in these pans with 150 g. of dough per loaf. Loaves of 600 to 750 cc. closely approximate standard commercial one-pound loaves in general appearance. As might be expected the pan proved to be a little too wide for loaves of very small volume and not wide enough for those with volumes above 800 cc. The break or shred of loaves baked in these pans appeared to be rather closely correlated with the relative strength of the flours. The strongest flours tend to give a large smoothly shredded break while weaker flours tended toward rough shreds and shell tops. Green or underdeveloped doughs yield loaves with neither break nor shred. The grain of the loaves tends to the spherical type, with small even pores for good-quality flours. The structure is more uniform than either the high- or the low-pan loaves.

P. P. Merritt has made studies of the intermediate-type pan in comparison with the older high-form pan. He gave special attention to the loaf volume. His findings are summarized in the tabulation below. He commented on the results as follows: "From this limited bake it seems that the intermediate-type pans give a considerable increase in loaf volume and that this increase may vary with the strength of the flour. The spread in loaf volume between the strongest



Flour	Volume in high pan	Volume in inter- mediate pan	Difference
	cc.	cc.	cc.
Strong spring	580	695	115
Intermediate spring	550	670	120
Low-protein hard winter	530	595	65

and weakest flours has been increased from 50 to 100 cc. It is considerably easier to place the molded loaf in the intermediate pan, uniformly, than in the narrow high-sided tins. This should reduce the individual's personal standard error although no work was done to establish this fact satisfactorily."

Earl Frank compared the new intermediate-form pan with the standard high-form pan. His results are tabulated below. His vari-

Flour	Volume in high pan	Volume in inter- mediate pan	Difference
	cc.	cc.	cc.
Low-protein hard winter	584	639	55
Medium-protein hard winter	662	739	77
Medium-protein hard spring	659	747	88
High-protein hard spring	690	780	90

ability was a little greater with the lower pans than with the high-form pans which he had been using for many years. He sums up his test as follows: "Characteristics of the crumb of the loaf were better for the loaves baked in the high pans. Cells were smaller and more elongated and the color whiter. We see no advantage in favor of the intermediate-type pans for our own laboratory's use."

G. Moen made a comparison of the intermediate pan with the high form pan with regard to effect of variation in absorption upon the loaf volumes. His data are shown below. From these data it appears that

Absorption	Dough condition	Volume in high pans	Volume in inter- mediate pans	Difference
%		cc.	cc.	cc.
60	Stiff	670	750	80
65	O.K.	695	740	45
70	Soft	725	730	5

the intermediate pan is much less critical of errors in judging absorption than is the standard high pan. The determination of the correct absorption is one of the most difficult tasks in the average laboratory and a pan less critical of small differences in absorption would expedite

much of the testing. Possibly this would reduce some of the large interlaboratory errors which now occur.

The intermediate-form pan was found by Claude Davis to give loaves with rougher breaks and more frequent shell tops than the Pioneer No. 1 pan. The grain was evenner in the intermediate pan than in the Pioneer No. 1.

The baking-pan problem involves certain fundamental considerations underlying the baking test itself. It is impossible to design a pan which will give optimum results with all flours and all variations of the basic baking test. A standard pan should be one which will aid in the differentiation of flours of all types. It might not be perfect for any one type of flour, and it would often be impractical for laboratories concerned with but a single flour type to use the standard pan. However a standard pan may be considered primarily as a reference standard for interlaboratory comparisons, in the same manner that the basic formula and time schedule are common points of departure. It appears to be impractical to attempt to develop a series of pans of graduated size to match the loaf volumes as a standardization procedure, so a pan suited to the median range of 550 to 750 cc., such as the intermediate type described here, may be best.

Whenever it is suggested that the baking pan could be improved there arise several objections. The first is that since extensive data have been accumulated with the older pan all would be lost if there should be a change. This reasoning is based on the false premise that baking values are absolute, like protein and ash, when actually all that can be expected of a baking test is relative information. When this is realized then the objections to variations in technique from season to season become of little importance. Our baking test is still in the developmental stage and consequently must not be crystallized as yet.

Another objection is that non-technical persons will not be able to interpret the results if a change is made in loaf shape or size. To this objection there is little to be said. After all it is the duty of the cereal chemist to interpret the baking results for the non-technical men, not *vice versa*. The third objection is the cost of new pans. If proper laboratory accounting methods are used this objection need not be made. Baking pans should not be carried on the property account. They are best handled as perishable supplies. If they must be carried as property, the depreciated life should not exceed five years. In a five-year life the annual cost per pan should not exceed 8 cents, which shows that baking pans are responsible for very little of the total laboratory costs.

In offering this new pan to the Association as a possible partial solution of the perennial baking-pan problem it is hoped that other

workers will give attention to this problem so that in the course of time a pan satisfactory to all may be evolved.

#### Acknowledgments

The author wishes to express his appreciation of the assistance given by Claude Davis in the design of the pan, and that of Mr. Ferris of the National Manufacturing Co. in the construction of the pans. The many baking tests carried out by Earl Frank of The International Milling Co., Paul P. Merritt of the Minnesota Agricultural Experiment Station, and George Moen of General Mills were of great assistance in determining the utility of this new intermediate pan.

#### Literature Cited

- Blish, M. J.  
1928 Standard experimental baking test. *Cereal Chem.* 5: 158-161.  
Davis, C. F., Leatherock, L. F., and Putnam, H. W.  
1936 Experimental baking pans. *Cereal Chem.* 13: 113-118.  
Herman, R. S., and Hart, V. M.  
1927 Some factors influencing the experimental baking test. *Cereal Chem.* 4: 157-183.  
Miller, H., and Whiteside, A. G. O.  
1938 A comparison of two sizes of baking pans on 100-gram flour doughs producing large loaf volumes. *Cereal Chem.* 15: 402-406.  
Moen, G.  
1935 Observations on grain and texture. *Cereal Chem.* 12: 78-83.

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### REPORT OF THE 1938-39 SUB-COMMITTEE ON METHODS OF TESTING CAKE FLOURS<sup>1</sup>

J. W. MONTZHEIMER, *Chairman*

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(Read at the Annual Meeting, May 1939)

The previous Subcommittee on Methods of Testing Cake Flour recommended consideration of commercial-type cake formulas, the preparation of photographic standards for grading interior and exterior characteristics, the further standardization of techniques, and the observation of pH in the finished cakes studied. As a result, this committee conducted an intensive search for a test cake baking scheme superior to the tentative A.A.C.C. formula.

Consideration of commercial-type test cake formulas was divided into two sections. The preliminary study was conducted by Coughlin and Wade,<sup>2</sup> who concluded that not even their detailed work was broad enough to suggest a "best" method for differentiating between cake flours. The results of their work, including the photographs of the cakes baked, were made available to the members of the committee.

One of these formulas, the A.A.C.C. formula and a modification of each, were then submitted to the committee for study. Four com-

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<sup>1</sup> Committee on Methods of Testing Soft Wheat.

<sup>2</sup> Francis J. Coughlin, and Donald Wade, Preliminary study of eight test methods, *Cereal Chem.* 17: 257-258, 1940.

mercial cake flours were used. These included a straight-grade white winter wheat flour, and short patent flours from red winter, white winter, and white spring wheats respectively. These flours showed the following ranges in analytical values: protein, 7.0% to 8.3%; ash, 0.33% to 0.42%; pH, 5.1 to 5.4.

The collaborators observed every possible precaution and recorded data on the batters and finished cakes similar to those collected by Coughlin and Wade.

The total scores assigned each flour when baked according to each formula were used as the committeemen's individual order of preference. It will be seen from Table I that these total scores in general

TABLE I

AGREEMENT BETWEEN COLLABORATORS ON RELATIVE VALUE OF DIFFERENT SAMPLES OF CAKE FLOUR AS REFLECTED BY TOTAL SCORES ASSIGNED CAKES BAKED FROM FOUR SELECTED FORMULAS

Collabo- rator	Basic A.A.C.C.	Modified A.A.C.C.	Formula No. 4	Formula No. 10	Basic A.A.C.C.	Modified A.A.C.C.	Formula No. 4	Formula No. 10
Flour L					Flour K			
A	1	2	2	2	3	3	1	3
B	1	1	1	1	2	2	2	4
C	1	2	1	2	3	1	2	1
D	1	1	2	1	3	3	2	3
E	2	3	1	3	1	1	2	2
Flour M					Flour J			
A	2	1	3	1	4	4	4	4
B	3	3	3	3	4	4	4	2
C	2	3	3	2	4	4	4	4
D	1	2	1	2	4	4	4	4
E	4	4	3	4	3	2	4	1

rated the flours in the order of *L*, *K*, *M*, and *J* from best to poorest. While individual differences in this order were reported, it is interesting to know that the averages of the collaborator's scores for cake volume lined up the flours in this same order.

As a matter of record, the A.A.C.C. formula was modified by the use of commercial baking powder in place of cream of tartar and soda and formula No. 4 was used both as given in Coughlin and Wade's report with ordinary hydrogenated shortening and as modified by additional liquid and special emulsified-type commercial cake shortening. It is believed that the regular A.A.C.C. formula and formula No. 4 gave the most consistent results by all collaborators.

The collaborative work again indicated the inadequacy of the present scoring system. Photographic standards for grading grain and shape of cake were prepared by Stamberg (1939),<sup>3</sup> who also offered constructive suggestions for handling the volume scoring data.

<sup>3</sup> Olof E. Stamberg, Standardization of the scoring of test cakes. Cereal Chem. 16: 764-768, 1939.

### Observations by Collaborators

Some of the observations and comments made by collaborators are recorded here for such value as they may have to others studying this problem.

Coughlin and Wade felt that the present scoring system placed too much emphasis on texture and grain and not enough on general eating and keeping qualities—another indication of defect in the present method. They point out that the color of a cake made by a creaming method is not always significant because contaminating metal may be ground from a tinned bowl. They have observed that results on yellow layer cakes may be unsymmetrical even with a balanced formula and ingredients of the highest quality because of the effects of hot areas and drafts in the oven.

Stokes and Track applied the seven formulas used by Coughlin and Wade and two from their own laboratory to two samples of cake flour. They felt that each of the formulas registered differences between the two flours. They pointed out that in the Coughlin and Wade data a number of cakes which appeared of equal merit in the photographs differed in final scores by the value assigned for total volume. Their view, shared by several committee members, is that volume scores should be recorded separately from the total score which in turn should represent the internal and external characteristics—grain, texture, moisture, and symmetry. They concluded that while more than one formula probably are required for testing the true baking characteristics of cake flour, it should be possible to set up a formula with sufficient variables in the proportions of sugar, shortening, liquid, and mixing to reflect baking qualities in a manner adequate for the requirements of the average baker.

Differences of opinion still exist among members of the committee as to the relative value of round layers and loaf cakes.

### Suggestions for Further Study

The committee makes the following suggestions which may serve to help future committees to arrive at a solution of the problem of properly differentiating between cake flours:

1. Two formulas (one rich and one lean) with appropriate variations may be required.
2. More detailed specifications for procedures must be developed and interpreted, including more specific directions for: make-up of batters; baking; standardization of baking conditions (moisture in oven, regulation of dampers, control of baking losses, determination of actual baking temperatures); cooling practices (time allowed, humidity conditions to be maintained); and volume measurements.

3. Observations should be made to determine the extent to which differences in mixer bowls (size and shape) influence results.

### Recommendations

The committee recommends: (1) that the system for scoring proposed by Stamberg (1939) be given careful collaborative study, and (2) that an effort be made to devise a method for measuring texture quantitatively.

### Errata

Through an oversight, the wrong dimensions for the loaf pan were given in the 1937-38 report (Montzheimer, *Cereal Chem.* **16**: 109). The recommended dimensions are: top  $8'' \times 4''$ ; bottom  $7\frac{1}{4}'' \times 3\frac{1}{4}''$ ; depth  $2\frac{1}{4}''$ . This is an oblong pan constructed of 4XXXX tinned iron.

### Acknowledgments

The chairman wishes to acknowledge the consistent assistance and thoughtful help given by members of the committee: F. J. Coughlin, H. V. Moss, R. W. Mitchell, O. E. Stamberg, W. E. Stokes, E. P. Walker, and L. D. Whiting, and by their associates. Further appreciation is due William R. Green, who baked cakes for exhibiting at the annual meeting.

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VOL. XVII

JULY, 1940

No. 4

**THE STATE OF WATER IN COLLOIDAL GELS: FREE  
AND BOUND WATER IN BREAD DOUGHS<sup>1</sup>**

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(Received for publication December 2, 1939)

In recent years considerable interest has been manifest regarding the state of water in both inorganic and organic colloidal systems. The systems investigated include such diverse materials as silica gels, lamp-black and water, cold-resistant wheat and insects, living cells, dairy products, frozen foods, fresh and dried vegetables, cooked meats, and other colloidal gels. Of these, dough is one in which the state of water has been little studied. Some work has been done on flour-and-water suspensions in which the ratio in one study was 5 parts of flour to 50 parts of water and, in another, approximately 15 parts of flour to 85 parts of water. The water content in such cases was far in excess of that found in bread doughs, in which the ratio is about 55 parts of flour to 45 parts of water.

Much consideration has been given the tendency of certain doughs to become less plastic with continued mixing. The ability of doughs made from certain flours to withstand mixing and to show little loss of plasticity has been determined. This characteristic is frequently considered in testing flours for quality. The cause of this change in plasticity is not known but is frequently attributed to "the breaking down of the gluten"—a statement which apparently has little demonstrated scientific basis. The possibility of a change in the state of the water in a flour-water system with change in degree of mixing has been recognized. Some work has been done in an endeavor to determine whether or not such a change occurs, but no definite conclusions have been reached.

It was the purpose of this investigation to study further the state of water in bread dough, employing an adaptation of a technique found useful in the study of colloidal gels in a related field.

<sup>1</sup> Paper No. 1758, Scientific Journal Series, Minnesota Agricultural Experiment Station. Condensed from a thesis presented by Gladys E. Vail to the faculty of the Graduate School of the University of Minnesota in partial fulfillment of the requirements for the degree of Doctor of Philosophy, July, 1938.



In this study, a modification of the cryoscopic method was used. In studying freezing-point depressions, Schofield and Botelho Da Costa (1935) found that when the degree of supercooling was lessened they obtained freezing-point depressions only about one-half the value of those obtained by the Bouyoucos method using the Beckmann technique. Rather recently, Alexander and Shaw (1937 and 1937a) and Alexander, Shaw, and Mulkenhirn (1937), as a result of the findings obtained by Schofield and Botelho Da Costa (1935), started work on soil-water relationships. Alexander and Shaw considered the criticism of Kistler (1936) and of Briggs (1932) concerning the use of the Beckmann technique in the freezing-point-depression determinations and they concluded that failure to obtain equilibrium was a major criticism of this method. In determining the dielectric constant of soils they conceived the idea of employing that physical property in determining the freezing-point depressions of soil. They believed that the great difference in the dielectric constant of water and of ice should give a sharp change in the capacitance of a condenser containing water as one of the components, at the freezing or melting point.

Alexander and Shaw (1937 and 1937a) suggested that one may study the freezing-point depression by determining either the melting point or the freezing point, allowing sufficient time for equilibrium to be established. In most of their reported work, melting points were determined.

There are a number of theories regarding the structure of colloidal systems which may or may not explain the change in viscosity of flour-water systems.

An early view of colloidal solutions held by Hatschek (1927) was that it contained independent particles, each surrounded by a thick film of solvent, which in effect increased the particle size and also the viscosity. This view is not popular with some of the recent workers with wheat-flour suspensions and doughs, who find it difficult to explain the loss in plasticity on this basis.

On the other hand, the "brush-heap" picture presented by Newton and Cook (1930), by Skovholt and Bailey (1935), and by others offers a means of visualizing differences of flour strength and also changes in plasticity under various conditions. The micellar structure of the flour-water system is favored by a number of workers. This theory offers a possible explanation of differences in plasticity. It pictures the micelles in various degrees of aggregation, so that without appreciable change in degrees of hydration they may entrap more or less water to give a more or less viscous flour suspension.

Probably the first study of the "bound water" in flour-water systems was made by Newton and Cook (1930), using suspensions.

These workers measured bound water of wheat-flour suspensions in an attempt to obtain an index of flour quality. This provided a simple means for direct measurement of the approximate hydration of the colloidal constituents. In this study, the workers found no significant differences in degree of hydration between strong and weak flours. The results seem opposed to those obtained with plasticity studies but the authors suggested that the "brush-heap" structure made up of interwoven micelles offers a means of visualizing differences in gluten which would affect the strength.

Skovholt and Bailey (1935) were among the early workers to consider the bound water of a dough rather than of a fluid system. They devised a technique whereby the freezing point of the dough being studied could be determined. The method was rapid and it was possible to make a determination within a few minutes. Although the readings were somewhat erratic, the authors considered that when an average was taken of all of the readings, satisfactory results were obtained. The results indicated that the calculated bound-water values were not significantly different as a result of variable mixing and were approximately the same in doughs containing each of the three flours studied. The average bound-water value obtained was 51.4% of the total water present.

These workers did find significantly lowered freezing points of all doughs with an increase in the mixing time. due, apparently, to increasing concentration of solute. They were able to show an increase in the reducing sugars present with increased mixing which paralleled the decrease in freezing point. This increase was sufficient to account for from 13% to 16% of the observed decrease in freezing point when comparing doughs mixed for 1 and for 10 minutes. The authors considered that a hydrolysis of starch would probably be accompanied by an increase in the amount of intermediate products. These in turn might have some effect on the freezing point of the system. There may have been a similar hydrolysis of the protein but this would be difficult to prove.

Skovholt and Bailey believed that reactions of this type, in addition to increasing the soluble material of the system, also required water, thus further increasing the effect on the freezing point.

### Experimental

In the present study, the purpose of which was to determine the state of water in doughs, it was proposed to determine the freezing or melting point of doughs without the supercooling characteristic of the Beckmann technique. The change in dielectric constant with the

melting or freezing of water as described by Alexander, Shaw, and Mulkenhirn (1937) was the basis for the work which follows.

The dielectric constant value was determined by measurement of the capacitance of a condenser under certain conditions. In this condenser or cell, the number of plates, the area of the plates, and the distance by which they were separated were fixed for each determination. The nature of the dielectric was the factor which varied, and accordingly the change noted in the capacity of the cell with change in temperature was due solely to the change in the physical state of the dielectric.

### Materials and Method Used

The materials remained the same throughout the study. A commercial red winter wheat flour containing 10.1% of protein and 11.6% of moisture was used. The flour was stored in a sealed container at  $5^{\circ} \pm 3^{\circ}\text{C.}$  during the experiment. Sufficient flour for one mixing was removed to a small can with a tight-fitting lid and taken to the laboratory to reach room temperature before weighing. Distilled water was mixed with the flour to make the dough. One lot of c.p. sucrose was used throughout. The crystals were large and were added to the water in this form.

To study the effect of the length of the mixing period on the state of water in the dough, 100 g. of the flour were weighed, 60 g. of distilled water added, and these mixed in the Hobart-Swanson mixer for 1-, 3-, and 10-minute periods. The attachment of the mixer made 112 revolutions per minute.

When studying the depression of the freezing point resulting from the addition of sucrose, the sugar and distilled water were weighed, mixed, and the mixture weighed. This was agitated gently until all of the sucrose was dissolved. The solution was weighed again and, since any loss of weight was due to evaporation, the solution was made up to weight by the addition of distilled water. The flour was weighed and the two mixed immediately. All dough preparations containing sucrose were mixed for three minutes.

As in the doughs mixed for different lengths of time, 100 g. of flour and 60 g. of water were used. The flour was found to contain 11.6% moisture. Calculations to determine the amount of sucrose to give a 0.125, 0.25, and 0.5 molal solution were made on the basis of 71.6 g. of water. The amount of sucrose used for the solutions was 3.062 g., 6.125 g., and 12.25 g., respectively.

The circuit used for determining change in capacitance in the special condenser was essentially that of Alexander, Shaw, and Mulkenhirn (1937) and is shown in Figure 1. *O* is the oscillator, giving off radio

frequency waves at a frequency of 1797.5 kilocycles per second.  $L$ ,  $C_s$ , and  $C_x$  form the receiving circuit, in which  $L$  is the inductance,  $C_s$  the calibrated condenser, and  $C_x$  the special condenser. The circuit was tuned to resonance with the oscillator.

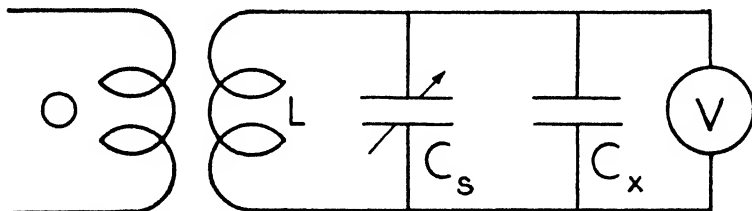


Fig. 1. Diagram of the circuit.  $O$  is the oscillator,  $L$  is the inductance of the receiving circuit,  $C_s$  is a calibrated condenser, and  $C_x$  is the special condenser in which the dough was placed.

The circuit was so designed that at a given capacity the receiver was "tuned" to the frequency of the transmitter. This is sometimes referred to as the resonance method of determining the dielectric constant. It is rather generally agreed that by this method small changes in conductance do not affect the capacitance readings. However, as pointed out by Alexander and Shaw (1937 and 1937a), an increase in the conductance of the dielectric, as occurred when the dough melted, resulted in a lowering of the sensitivity of the apparatus and unless the bias was set just right resulted in failure to measure small changes in capacitance of the special condenser.

The special condenser was made from a lusteroid 1.6 cm.-diameter test tube with the bottom cut off. This was inserted between two metal plates,  $9.6 \times 2$  cm., bent to fit the tube and cemented to a larger glass tube so that their positions with relation to each other were fixed. To the metal plates, copper lead wires were soldered.

To prepare the condenser for a determination the lusteroid tube was drawn full of dough. A metal tube 1.3 cm. in outside diameter with a tapering point on one end was forced through the dough. Five such preparations were usually made from each dough and the melting point determined in from one to three of these. In each instance, a period of 10 minutes elapsed from the time the water was added to the flour to the filling of the tubes. That is, the dough mixed 1 minute stood for 9 minutes, that mixed 3 minutes stood for 7 minutes, and that mixed 10 minutes was placed in the tubes immediately. In general, it required about 10 minutes to fill the lusteroid tubes and insert the metal tubes into the dough. Thus about 20 minutes elapsed between the addition of the water to the flour and the placing of the dough in the kerosene at approximately  $-16^\circ\text{C}$ .

As soon as the required number of tubes were ready, they were taken at once to the cold room, immersed in chilled kerosene, both at

approximately  $-16^{\circ}\text{C}.$ , and allowed to remain there until the melting point was to be determined. Then the tube to be used was taken from the kerosene, the kerosene emptied from the center metal tube, and the tube filled with water at approximately  $35^{\circ}\text{C}.$  This loosened the dough attached to the metal, the metal tube was readily withdrawn, and the dough remained frozen. The tube of dough was immediately inserted between the two metal plates, and the whole placed in a Dewar flask containing kerosene at about  $-16^{\circ}\text{C}.$  The wires from the metal plates came out on opposite sides of the Dewar flask. A stopper containing three openings was placed in the Dewar flask, and the special condenser was held in place about a half inch below the level

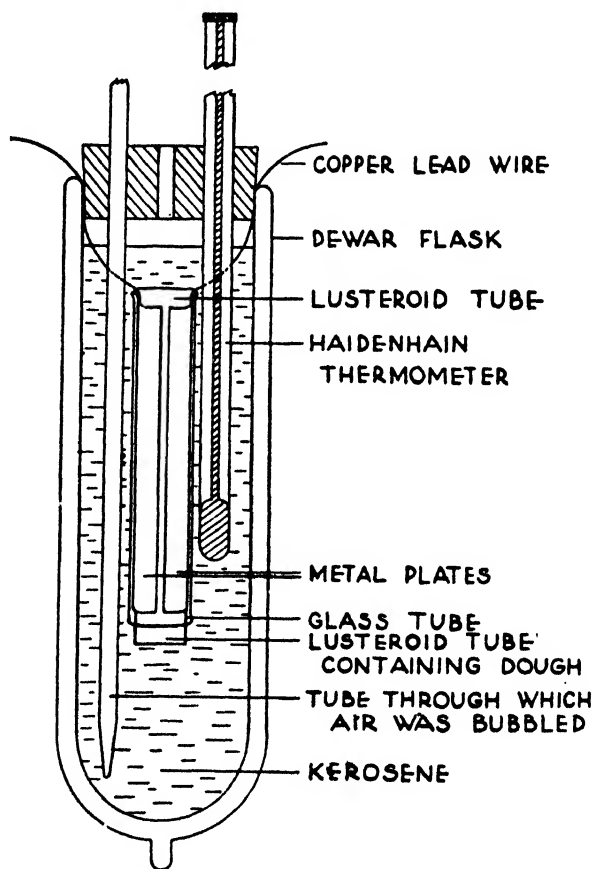


Fig. 2. Diagram of special cell, thermometer, glass tube, and stopper in place in Dewar flask.

of the kerosene. Through one opening in the stopper, a Haidenchain thermometer graduated in  $0.01^{\circ}\text{C}.$  with a range from  $-4.8^{\circ}$  to  $+1.0^{\circ}\text{C}.$  was inserted. A glass tube reaching almost to the bottom of the flask was inserted through the second opening. Cold dry air at a uniform pressure was passed through the glass tube to mix thoroughly the kerosene and thus maintain a uniform temperature throughout the flask.

The third opening was provided to permit free circulation of the air. No attempt was made to place the condenser in an exact position each time but the thermometer and condenser were adjusted so that they were close together near the center of the flask (Fig. 2).

The Dewar flask, with the condenser, thermometer, and glass tube in position, was then placed in an ice-salt bath. The special condenser containing the dough ( $C_x$  in Fig. 1) was connected in parallel with the calibrated variable condenser  $C_s$  of the receiver and the glass tube was connected with the source of the air under pressure. The receiver was tuned to the frequency of the oscillator and the temperature inside the flask was permitted to rise slowly. The change in capacitance of the special condenser ( $C_x$ ) with change in temperature was noted. This was done by adjusting the calibrated variable condenser ( $C_s$ ) to compensate for the change in the special condenser which in turn gave a method of measuring the change occurring in the special condenser.

The settings of the calibrated variable condenser were recorded approximately as follows for dough containing no sucrose: when first brought to the laboratory, then when the thermometer read, 7.5, 6.5, 5.0, 4.5, 4.0, 3.5, 3.0, 2.5, 2.0, 1.8, 1.7, 1.6, 1.4, 1.2, 1.1, 1.0, 0.9, 0.85, 0.8, 0.75, 0.7, 0.65, 0.6, 0.55, 0.5, 0.47, 0.44, 0.4, 0.37, 0.34, 0.3, 0.27, 0.24, 0.2, 0.17, 0.14, 0.1, 0.07, and 0.04 degrees below zero Centigrade, zero, and 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.8, and 1.0 degree above zero. When sucrose was added, the melting point was depressed so readings were taken at correspondingly lower points.

By plotting the values of the standard condenser against temperature a curve was obtained by means of which the melting point was determined.

Values of bound water were calculated by means of the widely used formula of Newton and Gortner (1922). This involves the use of freezing-point depressions with and without the addition of sucrose.

This formula assumes the formation of a hexahydrate of sucrose. Thus, for a 0.125 molal sucrose solution, 98.65% of the total water is not used in the formation of the sucrose hydrate; for a 0.25 molal sucrose solution, 97.3%; and for a 0.5 molal sucrose solution, 94.6%. The calculated values of the freezing point depressions ( $\Delta$ ) of the 0.125, 0.25, and 0.5 molal solutions are 0.236°, 0.478°, and 0.983°C., respectively. The formula for a 0.5 molal sucrose solution is:

$$\text{Percentage of bound water} = \frac{\Delta_a - (\Delta + K_m)}{\Delta_a - \Delta} \times 94.6$$

$\Delta$  = depression of freezing point of dough containing no sucrose;  
 $\Delta_a$  = depression of freezing point of dough containing sucrose;  
 $K_m$  = molal constant for the depression of the freezing point.

This is given in a different form by Skovholt and Bailey (1935):

$$\text{Percentage of bound water} = \frac{\Delta_0 - \Delta_c}{\Delta_0} \times 94.6$$

$\Delta_0$  = the observed difference in freezing point between doughs containing no added sugar and those containing added sugar sufficient to give a 0.5 molal concentration;

$\Delta_c$  = the calculated value for the freezing point depression due to 0.5 molal sucrose, assuming a hexahydrate formation.

The formulae of Newton and Gortner were recalculated by Grollman (1931), who proposed the following formula for use with a 0.5 molal sucrose solution:

$$\text{Percentage bound water} = \frac{\Delta_n - \left( \frac{1000}{946} \Delta + K_m \right)}{\Delta_n - \frac{1000}{946} \Delta} \times 94.6$$

### Results

Throughout this study the data were recorded as change in capacitance with change in temperature as indicated by the settings of the calibrated condenser necessary to bring the system to resonance. These data were plotted as capacitance against temperature, with the "break" in the curve indicating the melting point. As a check on the method and the apparatus, ice made from distilled water was melted.

The curve in Figure 3 shows the change in capacitance with change in temperature of distilled water. The first straight-line portion reading from the left demonstrates a rise in temperature with no appreciable change in dielectric constant of the medium as indicated by change in capacitance. The vertical line obtained by plotting change in capacitance against change in temperature indicates that the melting point has been reached. This is according to the theory of the change of dielectric constant of water as it melts. Melting was not quite complete before the temperature of the water immediately surrounding the thermometer began to rise. This is shown by the slight slope of the line which follows the vertical portion. This was verified by removing the Dewar flask from the ice bath and noting the small shell of ice remaining.

The dough preparations made by mixing the flour and water for 1-, 3-, and 10-minute periods had almost identical melting points, the average melting point for each being  $-0.3^\circ\text{C}$ . Plotting the data obtained by thawing the different doughs gave curves similar in shape.

The curves obtained by plotting change in capacitance against change in temperature for doughs mixed for one minute are shown in Figure 4.<sup>2</sup> Each curve represents the thawing of one tube of dough.

<sup>2</sup> Variations in the amount of dough in the tube and in the exact setting of the apparatus from time to time caused the total capacitance of the special condenser to vary. This accounts for the fact that the curves obtained by thawing a given series of doughs do not coincide.

Three different dough preparations are represented, duplicate samples having been used from one preparation. The horizontal line after the "break" indicates that thawing was complete. The length of time for melting and for the temperature to rise from  $-1^{\circ}$  to  $0^{\circ}\text{C}$ . are shown in

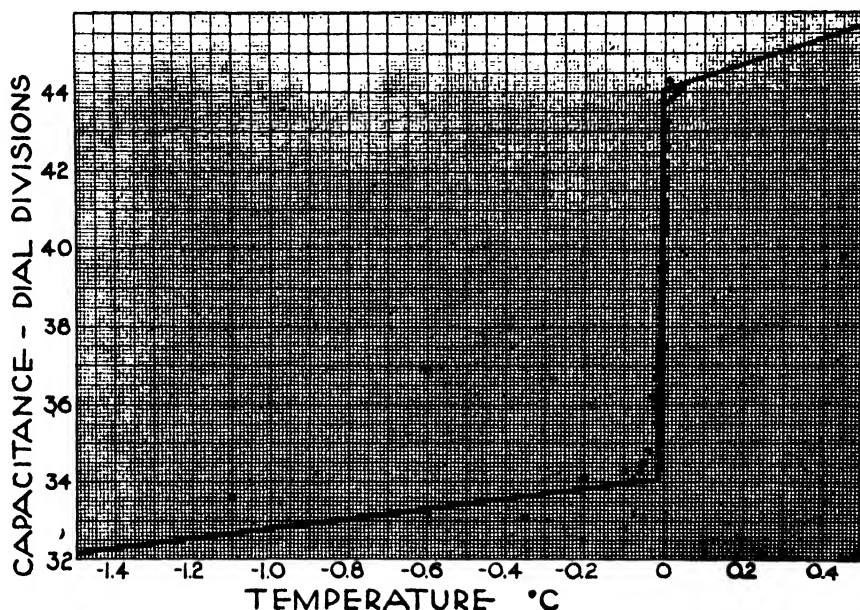


Fig. 3. Change in capacitance vs. temperature for distilled water during its elevation in temperature from the frozen to the fluid state.

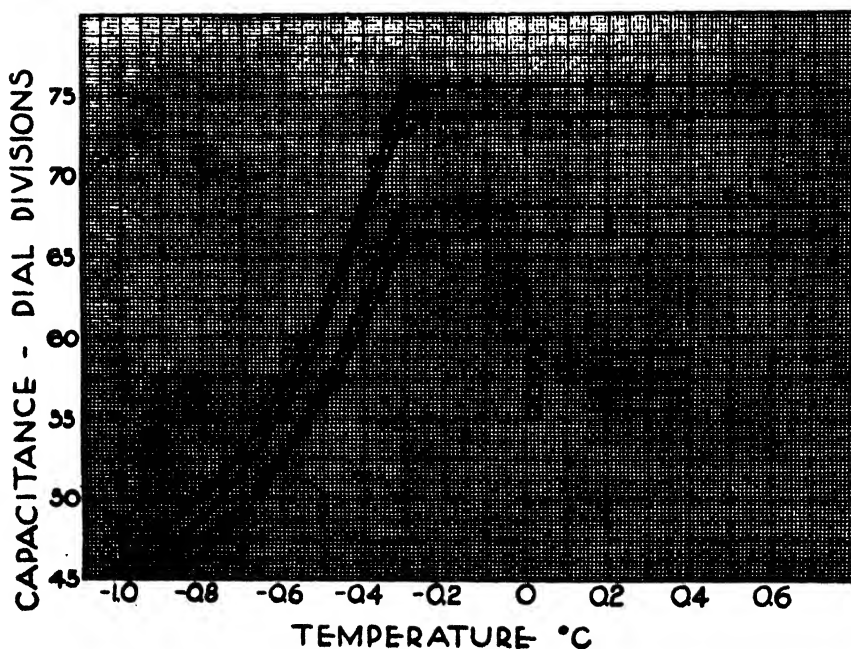


Fig. 4. Change in capacitance vs. temperature for doughs mixed one minute.



Table I. For complete thawing, a minimum of 4 hours was required. The significant range of temperature for these determinations was from  $-1^{\circ}$  to  $0^{\circ}\text{C}$ . Approximately 2 hours and 30 minutes was the minimum time in which the temperature was satisfactorily brought through this range.

TABLE I

SUMMARY OF THAWING DATA FOR DOUGHS MIXED ONE MINUTE—WITHOUT SUCROSE

Curve	Dough preparation No.	Total time of thawing	Time to rise from $-1^{\circ}$ to $0^{\circ}\text{C}$ .	Melting point— $^{\circ}\text{C}$ .
A	1	4 hrs. 6 min.	2 hrs. 27 min.	$-0.30$
B	2	4 hrs. 8 min.	2 hrs. 38 min.	$-0.30$
C	2	4 hrs. 30 min.	2 hrs. 39 min.	$-0.30$
D	3	4 hrs. 46 min.	2 hrs. 51 min.	$-0.30$
			Average	$-0.300$

The fact that there is no vertical portion in the curves obtained by melting the dough indicates that a solution and not water alone is being melted. Soluble materials in the flour have affected the freezing point of the water. As each increment of ice is frozen, the remaining solution is more concentrated and the freezing point is lowered. As the temperature is lowered more free water is frozen. When the process is reversed, the ice begins to melt. As the solution becomes more dilute, the melting point of the remaining ice is raised until finally melting is complete.

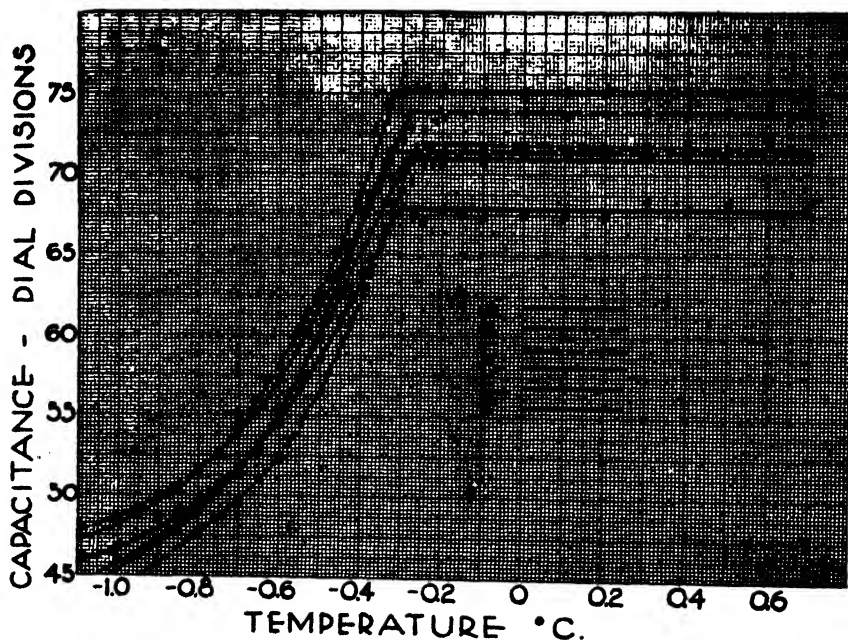


Fig. 5. Change in capacitance vs. temperature for doughs mixed three minutes.

Results of the plotting of data obtained by thawing doughs mixed for three minutes are recorded in Table II and in Figure 5. The satisfactory results obtained with shorter melting periods (curves *B* and *C*) indicate that possibly the dough mixed for three minutes thawed completely in less time than dough mixed for one minute. The results recorded are from three different dough preparations, using duplicate samples from each. The shape of the curves and the melting point do not differ significantly from those obtained with a one-minute mixing period.

TABLE II  
SUMMARY OF THAWING DATA FOR DOUGHS MIXED THREE  
MINUTES—WITHOUT SUCROSE

Curve	Dough preparation No.	Total time of thawing	Time to rise from -1° to 0°C.	Melting point—°C.
A	1	3 hrs. 37 min.	1 hr. 21 min.	-0.31
B	1	3 hrs. 15 min.	1 hr. 59 min.	-0.28
C	2	3 hrs. 18 min.	1 hr. 13 min.	-0.27
D	2	3 hrs. 34 min.	1 hr. 23 min.	-0.29
E	3	4 hrs. 55 min.	3 hrs. 18 min.	-0.32
F	3	4 hrs. 22 min.	2 hrs. 15 min.	-0.33
			Average	-0.300

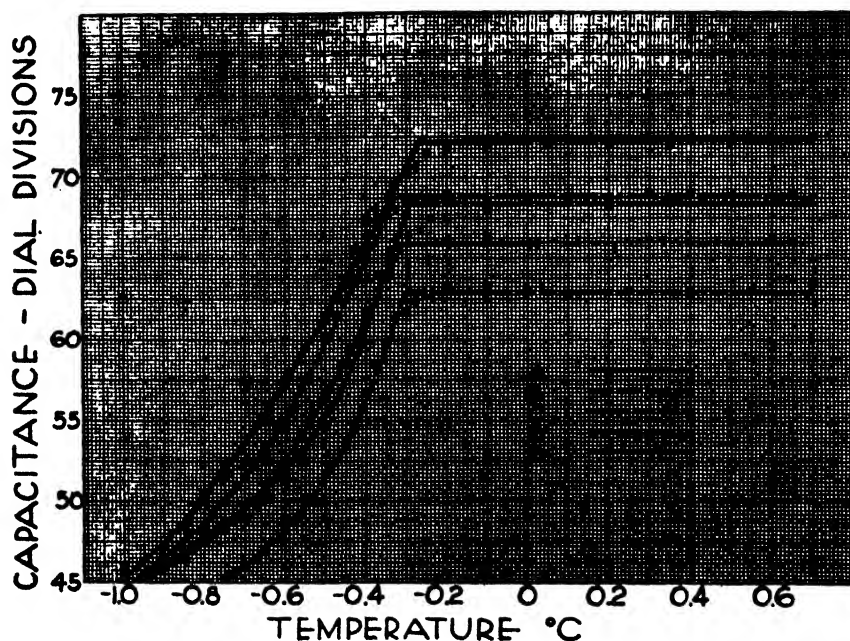


Fig. 6. Change in capacitance vs. temperature for doughs mixed ten minutes.

Figure 6 graphs the data obtained by thawing doughs mixed for a ten-minute period. These data are summarized in Table III. These represent two dough preparations using triplicate samples from one and

TABLE III

SUMMARY OF THAWING DATA FOR DOUGHS MIXED TEN MINUTES—WITHOUT SUCROSE

Curve	Dough preparation No.	Total time of thawing	Time to rise from $-1^{\circ}$ to $0^{\circ}\text{C}.$	Melting point— $^{\circ}\text{C}.$
A	1	4 hrs. 10 min.	2 hrs. 41 min.	-0.27
B	1	4 hrs. 32 min.	2 hrs. 45 min.	-0.33
C	1	2 hrs. 26 min.	1 hr. 37 min.	-0.30
D	2	4 hrs. 10 min.	1 hr. 11 min.	-0.30
E	2	7 hrs. 28 min.	2 hrs. 44 min.	-0.31
			Average	-0.302

duplicate from the other. The shape of the curves indicates that the thawing was complete in each instance. The melting period for one determination (curve C) was extremely short and the time required for a rise from  $-1^{\circ}$  to  $0^{\circ}\text{C}.$  was short in another of the determinations (curve D). The increase in mixing time gave a dough different in consistency from that obtained with less mixing. It may be that the smoother, more uniform dough resulting from the longer mixing time conducted the heat more efficiently, thus enabling it to reach a uniform temperature more quickly. Whether or not that is the explanation, it seemed that complete melting could be accomplished in less time with dough mixed for a longer period.

Addition of sucrose affected both the melting point and the shape of the curve. The results with a 0.125 molal sucrose solution are recorded graphically in Figure 7, representing duplicate samples from two different dough preparations. Not only was the melting point depressed but the rate of change in capacitance per unit change in temperature was less than that obtained when no sucrose was added. This tendency persisted on increasing the concentration of sucrose as is evident by comparing Figures 7, 8, and 9. With added sucrose, the time of thawing was decreased materially. The dough behaved more like the dough mixed for a longer period. The change in the shape of the curve was no doubt due to the smaller increment of water freezing at each temperature which resulted from the increased concentration of solutes.

The results with a 0.25 molal sucrose solution are shown in Figure 8. With increasing proportions of sucrose the dough thawed more rapidly and apparently temperature equilibrium was maintained with the more rapid rate of temperature increase.

Figure 9 shows the gentle slope of the curve obtained with 0.5 molal sucrose solution. The five sets of data involved two different dough preparations using triplicate samples from one and duplicate samples from the other.

Summaries of the results obtained with doughs mixed for three minutes are given in Table IV. These include the calculated "percent of bound water" computed from the melting-point data obtained by thawing the doughs mixed for three minutes, both with and without

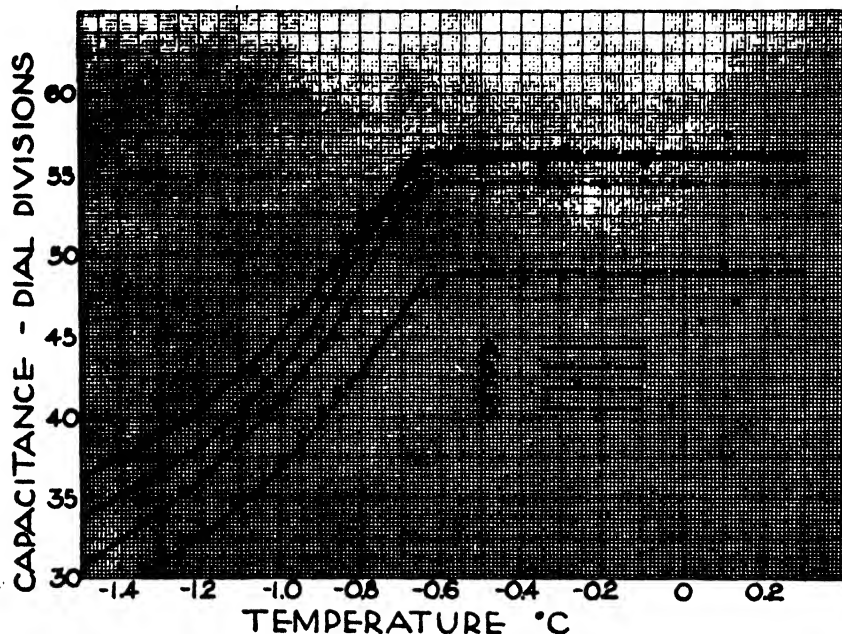


Fig. 7. Change in capacitance vs. temperature for doughs, 0.125 molal sucrose added.

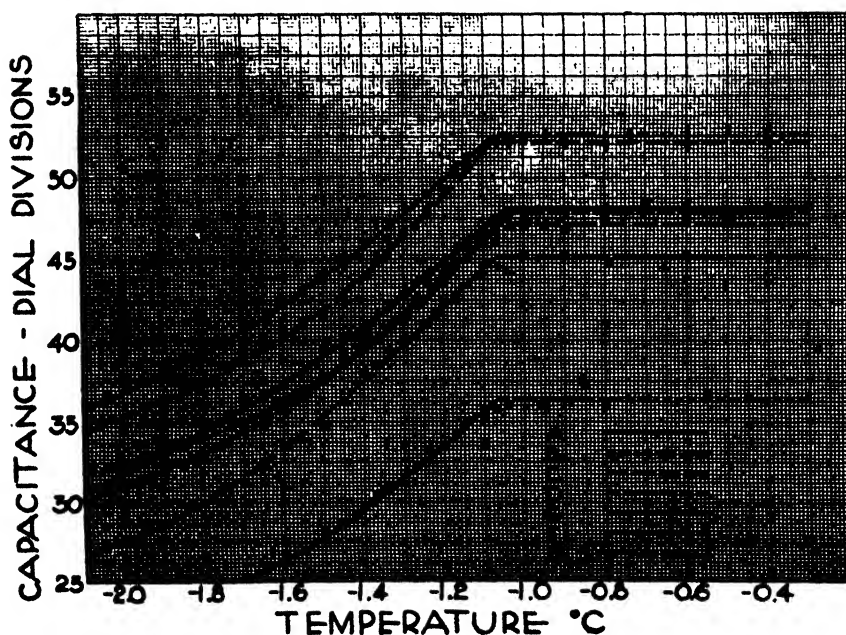


Fig. 8. Changes in capacitance vs. temperature for doughs, 0.25 molal sucrose added.

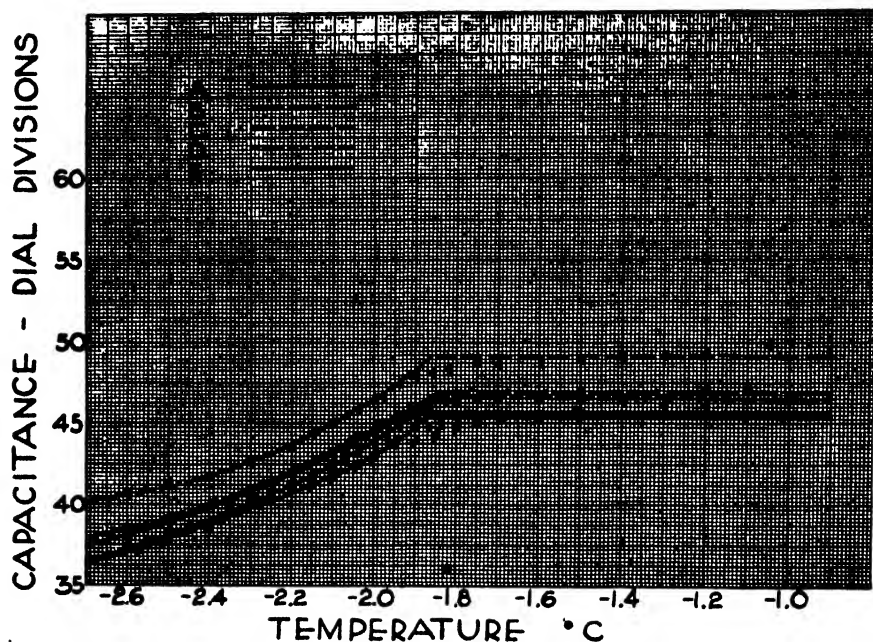


Fig. 9. Change in capacitance vs. temperature for doughs, 0.5 molal sucrose added.

the addition of sucrose. The formula of Newton and Gortner (1922), as given earlier, was used in calculating the percentage of bound water. The melting points obtained in this study were substituted for the freezing points indicated in the original formula, as the former were considered to be of the same value as the latter.

With a 0.125 molal sucrose solution:

$$\text{Percentage bound water} = \frac{0.665 - (0.3 + 0.236)}{0.665 - 0.3} \times 98.65 = 34.85$$

When a 0.25 molal sucrose solution was used:

$$\text{Percentage bound water} = \frac{1.064 - (0.3 + 0.478)}{1.064 - 0.3} \times 97.3 = 36.46$$

Using the results obtained with a 0.5 molal sucrose solution:

$$\text{Percentage bound water} = \frac{1.862 - (0.3 + 0.983)}{1.862 - 0.3} \times 94.6 = 35.06$$

Thus, using a 0.125, a 0.25, or a 0.5 molal sucrose solution, the results are in close agreement and there is no evidence that an increase of sucrose to give as much as a 0.5 molal solution tends to dehydrate or remove water from the flour particles.

TABLE IV

SUMMARY OF DATA FOR DOUGHS MIXED THREE MINUTES—WITH AND WITHOUT ADDED SUCROSE

Curve	Dough prepara- tion No.	Total time of thawing	Time to rise from -1° to 0°C.	Melting point—°C.	
NO SUCROSE ADDED					
A	1	3 hrs. 37 min.	1 hr. 21 min.	-0.31	
B	1	3 hrs. 15 min.	1 hr. 59 min.	-0.28	
C	2	3 hrs. 18 min.	1 hr. 13 min.	-0.27	
D	2	3 hrs. 34 min.	1 hr. 23 min.	-0.29	
E	3	4 hrs. 55 min.	3 hrs. 18 min.	-0.32	
F	3	4 hrs. 22 min.	2 hrs. 15 min.	-0.33	
			Average	-0.300	
Curve	Dough prepara- tion No.	Total time of thawing	Time to rise from -1.5° to -0.5°C.	Melting point—°C.	Bound water, %
0.125 MOLAL SUCROSE ADDED					
A	1	3 hrs. 19 min.	1 hr. 48 min.	-0.68	38.92
B	1	3 hrs. 41 min.	1 hr. 28 min.	-0.66	33.52
C	2	3 hrs. 29 min.	1 hr. 31 min.	-0.64	28.02
D	2	3 hrs. 10 min.	1 hr. 28 min.	-0.68	38.92
			Average	-0.665	34.85
Curve	Dough prepara- tion No.	Total time of thawing	Time to rise from -1.5° to -0.5°C.	Melting point—°C.	Bound water, %
0.25 MOLAL SUCROSE ADDED					
A	1	3 hrs. 33 min.	1 hr. 29 min.	-1.05	34.64
B	1	3 hrs. 21 min.	1 hr. 22 min.	-1.06	35.91
C	2	3 hrs. 4 min.	1 hr. 3 min.	-1.08	38.46
D	2	2 hrs. 42 min.	0 hr. 56 min.	-1.04	33.37
E	2	3 hrs. 27 min.	1 hr. 13 min.	-1.07	37.19
F	3	3 hrs. 19 min.	2 hrs. 0 min.	-1.08	38.46
G	3	3 hrs. 19 min.	1 hr. 32 min.	-1.07	37.19
			Average	-1.064	36.46
Curve	Dough prepara- tion No.	Total time of thawing	Time to rise from -2.5° to -1.5°C.	Melting point—°C.	Bound water, %
0.5 MOLAL SUCROSE ADDED					
A	1	2 hrs. 50 min.	44 minutes	-1.87	35.55
B	1	2 hrs. 35 min.	40 minutes	-1.85	34.34
C	1	2 hrs. 25 min.	36 minutes	-1.86	34.95
D	2	2 hrs. 44 min.	47 minutes	-1.85	34.34
E	2	2 hrs. 20 min.	63 minutes	-1.88	36.15
			Average	-1.862	35.06

### Discussion

Inasmuch as supercooling occurs in the cryoscopic method as it is usually employed, this method has been open to criticism. Schofield and Botelho Da Costa (1935) showed that by reducing the extent of the supercooling in soils they obtained freezing-point depressions in some

instances, only one-half the magnitude of those obtained by applying the ordinary Beckmann technique. A second criticism of the cryoscopic method is failure to obtain temperature equilibrium. The system is constantly changing as the "freezing point" is being established, and time is an important factor. The temperature at the freezing point cannot be maintained for an appreciable length of time by the methods ordinarily employed. It is also impossible to determine the rate of freezing or the quantity of water freezing at the different stages as might sometimes be desirable.

The modification of the cryoscopic method as used in this study has several advantages over the original Beckmann technique, especially as applied to viscous systems. Supercooling no longer occurs since a melting point rather than a freezing point is established. The rate of rise in temperature of the system may be controlled so that a uniform temperature is maintained throughout and any given temperature may be held for the length of time desired. Furthermore, an approximation of the amount of water freezing or melting at the different temperatures may be determined by studying the resulting curves.

Study of the curves obtained by plotting capacitance against temperature should indicate the rate of melting, whether a pure solvent or a solution is being melted, something of the concentration of the solution, and the melting point. The extent to which the set-up used in this study met the above specifications was checked by using distilled water (Fig. 3).<sup>3</sup> The slight slope in the left-hand portion indicates that a small portion is melting, and the almost vertical portion indicates that it is a pure solvent rather than a solution that is melting. At the frequency used, the dielectric constant of ice does not change measurably with temperature, according to Murphy (1934). The point at which the great increase in capacitance occurs is, then, the melting point of the ice.

When the dough preparations were melted (Figs. 4, 5, and 6), the slope of the curve indicated that a solution was melting. The addition of sucrose (Figs. 7, 8, and 9) resulted in a greater slope which tended to flatten out with increased concentration of sugar.

The melting point of doughs as indicated by the curves is, if anything, slightly high. There was a tendency for the change in capacitance to lag behind the change in temperature whenever the rate of rise in temperature was not sufficiently slow. Accordingly, it appears that the minimum readings obtained are probably more nearly those of the true melting points than are the average readings.

When the formula of Grollman (1931) was used the results were

<sup>3</sup> It was impossible to use a shell of ice as thin as that of the dough because of the nature of ice; neither could it be thawed directly in the kerosene as was the dough. Hence, with this special condenser it was never possible to obtain complete melting of the ice before some rise in temperature occurred.

34.15%, 35.78%, and 34.41% bound water, respectively, for the 0.125, 0.25, and 0.5 molal sucrose solution. These differ only about 0.7% from the values obtained using the formula of Newton and Gortner (1922).

Thus using different concentrations of sucrose, no consistent, appreciable difference in bound water is shown whether one uses the formula of Newton and Gortner or that of Grollman.

That the process of freezing does not alter the dough so that the melting point differs significantly from the freezing point may be assumed from the work of Skovholt and Bailey (1935) which showed that "many replicated freezings and thawings of dough systems indicated no significant trend in successive values."

Robinson (1931) found in his work with insects that, once frozen, there was no apparent change in the state of water and determinations could be made as desired. This appeared to be true in this work on flour-water systems also, although none of the doughs were kept for more than a week.

With the exception of the work of Skovholt and Bailey (1935), previous determinations of bound water in flour-water systems had been made on systems containing a high percentage of water. In view of other work on bound water using gelatin, gum acacia, and other colloids, it seems highly probable that a system containing a high proportion of water would give very different percentages of bound water from one containing a low proportion. It would seem, then, that the results of the present study could best be compared with those of Skovholt and Bailey, whose work more closely approximates this research than any other. These workers used doughs made of flour and water mixed for varying lengths of time with and without the addition of sucrose. In every instance the freezing points of the doughs were much more depressed in the work of Skovholt and Bailey than in the present study. This great difference in the depression of the freezing point is undoubtedly due to the different methods used for the determinations. Since Schofield and Botelho Da Costa (1935) showed that decreasing the degree of the supercooling decreased the magnitude of the depression of the freezing point, eliminating supercooling entirely would undoubtedly have a similar effect. Skovholt and Bailey found it impossible to prevent supercooling with the method they used.

A significant difference in the freezing-point depressions of dough mixed for 1-, 2-, 3-, 4-, and 10-minute periods was also found by Skovholt and Bailey. This was attributed to the formation of soluble materials in the dough rather than to changes in the state of the water. They found no significant differences in percentage of bound water as calculated from the freezing point using 0.125, 0.25, and 0.5 molal



sucrose solutions. They did find an average of approximately 51.4% of the total water present as bound water, whereas in this study the average was 35.5%. Calculating this as hydration value, "that is, the amount of water held as bound per unit weight of solid material" which Skovholt and Bailey said may be a better method for comparison, one finds in this study an average total hydration of 28.6% as compared with 43.2% given by Skovholt and Bailey for the flour which had the protein value nearest that of the flour used here.

Comparisons with the results of Newton and Cook (1930) and of Kul'man and Golosova (1936) are difficult. Newton and Cook used suspensions of approximately 15% concentration and Kul'man and Golosova used 5 g. of absolutely dry flour in 50 g. of water. Hence, in both instances the percentage of water was exceedingly high as compared with that in doughs. If one accepts the prevailing idea that "bound water" is a relative term and that, as some workers have shown, the percentage of "bound water" is dependent upon the concentration of colloid in the system and upon the method used, one could hardly expect complete agreement in the results of the different workers with flour-water systems. Newton and Cook studied their suspensions by the cryoscopic method, and Kul'man and Golosova by Dumanski's refractometric method.

These workers agree with each other and with Skovholt and Bailey in finding no great difference in the water-binding capacity of weak and strong flours, but, as far as the percentage of bound water is concerned, they disagree with each other.

For winter wheat flour, durum wheat flour, and spring wheat flour, Kul'man and Golosova found from 44.4% to 54.4% of water bound or a total hydration of from 44.4% to 54.4%. Newton and Cook found only about 2% of the total water to be in the form of bound water or a hydration of the flour of about 11%. However, Newton and Cook did little work with untreated flour-water suspensions because of the relatively large errors involved.

Newton and Cook believed that bound water is a measure of true hydration. Kul'man and Golosova stated that there is a direct relationship between water-absorbing capacity of flour and its water-binding capacity.

The present study was not carried far enough to show the percentage of bound water at different levels of mixing. It does show no significant difference in the shape of the curves and in the freezing point for the doughs mixed for 1, 3, and 10 minutes. This does not agree with the work of Skovholt and Bailey, which showed a decrease in the freezing point with increase in mixing. This decrease was partially accounted for by the increase in soluble sugars. If there were

an appreciable increase in the amount of soluble materials in the dough preparations used in this study, this should be shown in the shape of the curve as well as in the melting point. The effect of the increased concentration of solute is clearly shown by the results obtained when sucrose was added.

Skovholt and Bailey made "all determinations that involved studies on the effect of mixing, immediately after the completion of mixing." They were able to remove the dough from the mixer, prepare it for and place it in the freezing bath in an elapsed time of one to two minutes, which means that determinations were started on doughs approximately 3, 4, 5, 6, and 12 minutes after the beginning of the mixing period. In the present study, approximately 20 minutes elapsed from the beginning of mixing to the placing of the dough in the cold kerosene. Steller, Markley, and Bailey (1935) had previously shown that over 70% of the total reducing sugar produced in a one-hour period is formed in the first 15 minutes. The doughs in the study here reported were at room temperature for approximately 20 minutes, sufficient time, according to Steller *et al.*, for the formation of considerable reducing sugar. Since each dough preparation was at room temperature for the same length of time, it seems probable that approximately the same percentage of reducing sugar may have been formed regardless of the mixing period.

The fact that there was no difference shown in this work in the freezing points of doughs mixed for different lengths of time, but of the same age when frozen, indicates no change had occurred in the state of water in the dough in consequence of variations in the degree of mixing. This is in agreement with the results of other workers who believe the "break-down" in bread doughs with overmixing must be accounted for by some means other than by changes in the hydration of flour particles. This "break-down" may result from changes in the "brush-heap" structure in which Newton and Cook (1930) as well as other workers found a possible explanation. As pointed out earlier, the idea of interwoven micelles offers a means of explaining the difference in the strengths of flours. Strong flours may be no more hydrated than weak flours, as the results of different workers indicate, but the difference in the aggregation of the micelles may result in different amounts of water being entrapped. Hence, strong flours may adsorb more water and thus have a higher initial viscosity than weaker flours. Changes in viscosity with age or with prolonged mixing may be the result of the squeezing out of the entrapped water due to shrinkage of the micelles or to decrease in aggregation of the micelles. This would mean no change in the amount of water "bound" and is in accord with the results obtained.

### Summary

A method for determining the freezing or melting point of a system was devised which involved observing the changes in dielectric properties in progressing from the frozen to the melted state. The method is such that the system is in equilibrium and thus the stages in the freezing or thawing process may be observed. It is believed to be a suitable method for use in studying the state of water in highly viscous or plastic systems such as doughs.

Freezing points were determined for doughs mixed for 1-, 3-, and 10-minute periods. No significant differences were found. There was no indication of increase of solute with increase in mixing period under the conditions of dough treatment involved.

Average bound-water values for the dough mixed for three minutes were calculated. Calculations were based upon the depression of the freezing point of the doughs containing no sucrose and 0.125, 0.25, and 0.5 molal sucrose solutions. The calculated percentages of bound water at the three levels were 34.85, 36.46, and 35.06, respectively.

The average calculated bound water was 35.5% and the hydration capacity was 28.6% when calculated as bound water held per unit weight of dry matter.

### Literature Cited

- Alexander, L. T., and Shaw, T. M.  
1937 Determination of ice-water relationships by measurement of dielectric constant changes. *J. Phys. Chem.* **41**: 955-960.  
1937a A method for determining ice-water relationships by measurements of dielectric constant changes. *Nature* **139**: 1109-1110.
- Alexander, L. T., Shaw, T. M., and Mulkenhirn, R. J.  
1937 Detection of freezing point by dielectric measurements. *Proc. Soil Sci. Soc. Am.* **1**: 113-119.
- Briggs, D. R.  
1932 Water relationships in colloids. II. "Bound" water in colloids. *J. Phys. Chem.* **36**: 367-386.
- Grollman, A.  
1931 The vapor pressures of aqueous solutions with special reference to the problem of the state of water in biological fluids. *J. Gen. Physiol.* **14**: 661-683.
- Hatschek, E.  
1927 The apparent viscosity of colloidal solutions. *J. Phys. Chem.* **31**: 383-392.
- Kistler, S. S.  
1936 The measurement of "bound" water by the freezing method. *J. Am. Chem. Soc.* **58**: 901-907.
- Kul'man, A. G., and Golosova, O. M.  
1936 Bound water in bread making. *Cereal Chem.* **13**: 202-217.
- Murphy, E. J.  
1934 The temperature dependence of the relaxation time of polarizations in ice. *Trans. Electroch. Soc.* **65**: 133-142.
- Newton, R., and Cook, W. H.  
1930 The bound water of wheat flour suspensions. *Can. J. Research* **3**: 560-578.
- Newton, R., and Gortner, R. A.  
1922 Method for estimating hydrophylic colloid content of expressed plant tissue fluids. *Bot. Gaz.* **74**: 442-446.

- Robinson, W.  
1931 Free and bound water determinations by the heat of fusion of ice method. *J. Biol. Chem.* **92**: 699-709.
- Schofield, R. K., and Botelho Da Costa, J. V.  
1935 The determination of the pF at permanent wilting and at the moisture equivalent by the freezing point method. *Trans. Third Intern. Congr. Soil Sci.*, pp. 6-10.
- Skovholt, O., and Bailey, C. H.  
1935 Free and bound water in bread doughs. *Cereal Chem.* **12**: 321-355.
- Steller, W. R., Markley, M. C., and Bailey, C. H.  
1935 Factors affecting the diastatic activity of wheat flour. *Cereal Chem.* **12**: 268-275.

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## GAS PRODUCTION IN YEAST FERMENTATION AND ITS APPLICATIONS. I. VOLUMETRIC VS. MANOMETRIC METHODS FOR THE DETERMINATION OF GAS PRODUCTION

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(Received for publication December 18, 1939)

A fermenting dough is characterized at least in part by the formation and liberation of carbon dioxide. It is apparent that both the total number of moles of gas produced and the time rate of their production are in some way a rather complex function of the yeast, the substrate, and the state of the system. This functional relationship is of supreme interest.

The study of gas production may be expected to yield information concerning not only the substrate (flour and ingredients other than yeast), and the yeast, but concerning the functional relationship itself. With this concept undoubtedly in mind, Bailey and Johnson (1924) and Bailey (1939) applied and improved a volumetric technique for the study of gas production under conditions of relatively constant pressure. Blish, Sandstedt, and Astleford (1932) and Sandstedt and Blish (1934) on the other hand adopted a manometric technique, that of measuring changes in pressure at relatively constant volume.

None of these workers, however, has published a comparison of the inherent errors, the complexity of applying necessary corrections, or the care required in maintenance of equipment in the respective methods. It is the purpose of this paper to attempt a detailed study of these factors.

### Effects of Atmospheric Pressure

*The manometric method.*—A schematic diagram of the apparatus is given in Figure 1. *A* represents the vessel of volume *V* in which the

fermenting dough of non-gaseous volume  $v^*$  is placed.  $B$  indicates a simple mercury manometer of bore  $d$ . That segment of the left arm of the manometer between the mercury surface and the vessel  $A$  possesses an internal volume  $v'$ , and  $h$  signifies the difference in height

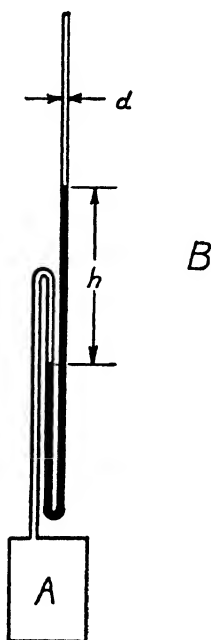


Fig. 1. Schematic diagram of manometric instrument.

between the mercury in both arms of the manometer—that is, the difference in pressure, say in millimeters of mercury, between the interior and the exterior of the vessel  $A$ .  $A$  is assumed immersed in a water bath at constant absolute temperature,  $T_{st}$ .

Throughout this paper we will adopt the following subscripts:  $st$  to represent standard conditions of pressure and temperature;  $s$  to represent the magnitude of the variable concerned at the time of sealing the equipment;  $r$  to represent the magnitude of the variable at the time a reading is taken. Let the atmospheric pressure be symbolized by  $P$  and let  $p$  represent the pressure of the gas produced by fermentation. For the sake of simplicity we will assume the gas law to hold. A more precise treatment might be attempted but the principles involved would remain unchanged.

At some time after sealing, consider  $n$  moles of carbon dioxide produced. Since the gas is assumed confined at constant free volume,  $V' = V + v' - v^*$ ,  $n$  is proportional to the resulting pressure,  $p_r$ ; that is

$$n = \frac{V'}{RT_{st}} p_r \quad (1)$$

where  $R$  is the gas constant in cubic centimeters—millimeters of mercury.

But:

$$p_r = h + P_r - P_s \quad (2)$$

where  $P_s$  is the partial pressure within the vessel due to atmospheric pressure at the time of sealing and  $p_r$  is the partial pressure of the carbon dioxide formed by fermentation during the interval sealing to reading. Obviously if there is no change in  $P$  during the course of measurement,  $\Delta P = P_r - P_s = 0$ , and  $n$  is simply proportional to  $h$ . If this condition is not fulfilled then an appropriate correction must be made to  $h$  if the proportionality of  $n$  to  $h$  is to be preserved. This correction is additive and is equal to  $\Delta P$ ; that is, the percentage correction in  $h$  associated with a given percentage change in  $P$  is equal to  $100\Delta P/h$ . It is to be noted that  $P_{st}$  does not occur in any of these relationships, and that therefore the manometric method is independent of any arbitrarily selected standard state of pressure so long as one does not calculate the volume corresponding to any given  $h$ . We may combine equations 1 and 2 to

$$n = \frac{V'}{RT_{st}} (h + P_r - P_s) \quad (3)$$

But

$$n = \frac{v_{st}}{RT_{st}} P_{st} \quad (4)$$

where  $v_{st}$  represents the volume of carbon dioxide under conditions of standard temperature and pressure, corresponding to the  $n$  moles liberated. Combining equations 3 and 4 we finally obtain:

$$v_{st} = \frac{V'}{P_{st}} (h + P_r - P_s) \quad (5)$$

where  $h$  and  $P$  are expressed in millimeters and  $v_{st}$  and  $V'$  in cubic centimeters.

*The volumetric method.*—Figure 2 presents a schematic diagram of the apparatus.  $A$  represents a vessel of any convenient volume  $V$  in which the fermenting dough of non-gaseous volume  $v^*$  is held.  $B$  is a rubber tube of internal volume  $v'$  connecting the vessel  $A$  to a Hempel gas burette  $C$ .  $D$  is some liquid preferably of low density, of low vapor pressure, and which does not absorb carbon dioxide.  $E$  is a leveling bulb so held that the liquid both in the burette and in the leveling bulb is at an identical level whenever a reading of the volume of gas produced is made.  $F$  is a rubber tube connecting burette to leveling bulb.  $A$  is assumed immersed in a constant temperature water bath at  $T_{st}$ .

The fixed total volume of air confined in the system at the time of sealing is then

$$V' = V + v' - v^* \quad (6)$$

It is this residual air in the system that complicates the corrections in the volumetric method. For if the atmospheric pressure changes from

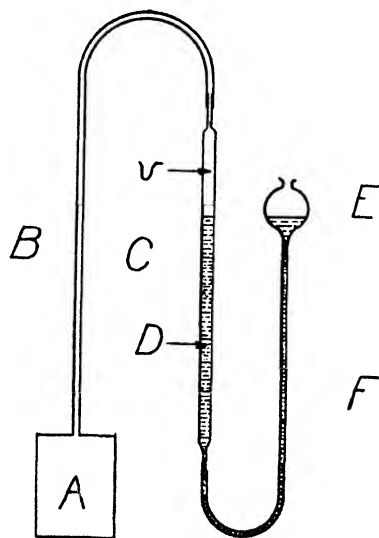


Fig. 2. Schematic diagram of volumetric instrument.

time of sealing to time of reading then the residual air itself will suffer a change of volume thereby causing an error in the volume of carbon dioxide  $v_r$  read from the burette. That is

$$V' + v_s = \frac{P_r}{P_s} (V' + v_r) \quad (7)$$

where  $v_s$  is the volume corrected for this error

$$v_s = \frac{P_r - P_s}{P_s} V' + \frac{P_r}{P_s} v_r \quad (8)$$

But

$$v_{st} = \frac{P_s}{P_{st}} v_s \quad (9)$$

where  $v_{st}$  is the reading corrected to standard pressure. Therefore:

$$v_{st} = \frac{P_r - P_s}{P_{st}} V' + \frac{P_r}{P_{st}} v_r \quad (10)$$

It is interesting to compare this relationship to equation 5, the final

expression for correcting the manometric reading for changes in atmospheric pressure. We rearrange equation 5 slightly for this purpose:

$$v_{st} = \frac{P_r - P_s}{P_{st}} V' + \frac{V'}{P_{st}} h \quad (5')$$

We see here that the manometric method possesses a tangible advantage over the volumetric. For when  $P_r = P_s \neq P_{st}$  the manometric method requires no corrections while the volumetric method requires one; and for all other cases where  $P_r \neq P_s \neq P_{st}$  corrections for the manometric method still require one less operation.

### Effects of Room Temperature

*The manometric method.*—Conventionally pressure is expressed in terms of millimeters of mercury and hence we are able to write  $p_r = h$ . But since in actual units of pressure  $p_r$  is expressed in dynes per square centimeters:

$$p_r = h\delta g \quad (11)$$

where  $\delta$  is the density of mercury at the temperature under consideration and  $g$  is the acceleration due to gravity. For a given value of  $p_r$ :

$$\frac{100\Delta h}{h} = -\frac{100\Delta\delta}{\delta} \quad (12)$$

and the percentage variation in  $h$  is equal to the percentage variation in  $\delta$  due to changes in room temperature. But a change of even  $100^\circ\text{C}$ . produces less than a 2% change in  $\delta$ . Therefore we may consider the manometric method free from errors of this sort.

*The volumetric method.*—Variations in room temperature during the course of the determination may be considered the most significant source of error in the volumetric procedure. One must view the total volume of carbon dioxide and air confined in the connecting tube  $B$  and in the gas burette  $D$ . Since the internal volume of the connecting tube  $B$  is  $v'$ , the volume of gas and air affected by variations in absolute room temperature,  $T$ , is equal to  $v_r + v'$ . We may write

$$v' + v_s = \frac{T_s}{T_r} (v' + v_r) \quad (13)$$

where  $v_s$  is the reading corrected to the temperature at sealing

$$v_s = \frac{T_s - T_r}{T_r} v' + \frac{T_s}{T_r} v_r \quad (14)$$

But

$$v_{st} = \frac{T_{st}}{T_s} v_s \quad (15)$$



where  $v_{st}$  is the reading corrected to standard temperature. Therefore:

$$v_{st} = \frac{T_{st}}{T_s} \left( \frac{T_s - T_r}{T_r} \right) v' + \frac{T_{st}}{T_r} v_r \quad (16)$$

Consider the first term to the right of the equality sign. It may be reduced to relative insignificance in two ways, firstly by making  $v'$  sufficiently small through use of short tubing of very small bore, and secondly by controlling fluctuations in room temperature. Nevertheless, we must consider that the volumetric procedure again falls somewhat behind the manometric procedure insofar as dependence upon variables is concerned.

We compare the final relationships for both methods:  $v_{st}$  symbolizes the measurement of either  $h$  or  $v_r$  as the case may be, corrected to conditions of standard pressure and temperature.

The manometric method:

$$v_{st} = \frac{P_r - P_s}{P_{st}} V' + \frac{V'}{P_{st}} h \quad (5')$$

The volumetric method:

$$v_{st} = \frac{T_{st}P_s}{T_sP_{st}} \left[ \frac{P_r - P_s}{P_s} V' + \frac{T_s - T_r}{T_r} v' + \frac{P_r T_s}{P_s T_r} v_r \right] \quad (17)$$

### Effects of Size of Fermentation Vessel

*The manometric method.*—As a starting point we may rewrite

$$v_{st} = \frac{h + P_r - P_s}{P_{st}} V' \quad (5)$$

Let us hold all conditions but  $V'$  constant and calculate what effect variation in  $V'$  will have upon a given  $v_{st}$  corresponding to  $n$  moles of carbon dioxide.

$$\Delta v_{st} = \frac{h + P_r - P_s}{P_{st}} \Delta V' \quad (18)$$

Dividing by  $v_{st}$  and multiplying by a hundred:

$$\frac{100\Delta v_{st}}{v_{st}} = \frac{100\Delta V'}{V'} \quad (19)$$

Or the percentage error induced in  $v_{st}$  by changing from one experimental system to another is equal to the percentage variation in  $V'$  in going from one to the other. We may conclude that the percentage variation in the  $V'$ 's among a group of manometric systems should not exceed the percentage variations among a series of replicate determinations in any one of these systems. The restrictions laid down by this

rule are somewhat greater than are at first apparent because one must remember that

$$V' = V + v' - v^* \quad (6)$$

and that  $v^*$  has been defined as the non-gaseous volume of the fermenting dough. This definition may well be broadened somewhat to include the non-gaseous volume of any object or substance placed in the fermentation vessel. Therefore, if as is sometimes done, caustic soda or salt solution is placed within the vessel, it is important that the same volume of liquid always be used. Theoretically, the same volume of dough should be used, if consistent results are to be obtained. The criterion for the amount of care to be used in employing these considerations is again the percentage variation in  $V'$  as defined above that may be tolerated without causing errors in either  $h$  or  $v_{st}$  greater than are warranted by the reproducibility of the method.

It is now apparent that measurements obtained with different-sized doughs are not directly comparable; that is, readings obtained say with a 5-g. dough are not equal to half of the corresponding readings obtained with a 10-g. dough. Rigidly considered, all doughs studied by this method should be of identical non-gaseous volume.

Another source of error depending upon the bore of the manometer tube may be mentioned. This error is unimportant if the necessary precautions are taken. For any one manometric system, as  $h$  increases  $v'$  increases by an amount equal to

$$\Delta v' = \Delta V' = \frac{h\pi d^2}{4} \quad (20)$$

If for example one wishes to keep this error at less than 1% then

$$\frac{100\Delta V'}{V'} = 1 = \frac{100h_m\pi d^2}{4V'} \quad (21)$$

and

$$d < \sqrt{\frac{4V'}{100h_m\pi}} \quad (22)$$

$$d < 0.113 \sqrt{\frac{V'}{h_m}} \quad (23)$$

If, for example, we take  $V' = 200$  cc., and a maximum  $h_m = 400$  mm. Then:

$$d < 2.53 \text{ mm.} \quad (24)$$

Or more generally, for any desired percentage error in  $V'$  or the corresponding percentage error in  $h$  or  $v_{st}$  for any maximum  $h_m$  and any  $V'$ :

$$d < \sqrt{\frac{4V'}{100h_m\pi} \left( \frac{100\Delta h}{h} \right)} \quad (25)$$

where  $\frac{100\Delta h}{h}$  is the permissible percentage error in  $h$  and  $h_m$  is the maximum value that  $h$  may be expected to attain.

*The volumetric method.*—Consider the analogue of equation 5:

$$v_{st} = \frac{T_{st}P_s}{T_sP_{st}} \left[ \frac{P_r - P_s}{P_s} V' + \frac{T_s - T_r}{T_r} v' + \frac{P_r T_s}{P_s T_r} v_r \right] \quad (17)$$

It is immediately apparent that errors induced by variations in either  $v'$  or  $V'$  are of a secondary nature; that is, neither  $v'$  nor  $V'$  is directly connected to the measured quantity  $v_r$ , but rather to the temperature and pressure corrections respectively. That is, this method is completely independent of vessel size in the absence of significant pressure and temperature corrections. When these are present, however, we may write for the dependence of  $v_{st}$  upon fluctuations in  $v'$  for any given set of conditions represented by  $T_s$ ,  $T_{st}$ ,  $T_r$ ,  $P_s$ ,  $P_{st}$ ,  $P_r$ , and  $v_r$ :

$$\Delta v_{st} = \frac{T_{st}P_s}{T_sP_{st}} \left[ \frac{T_s - T_r}{T_r} \right] \Delta v' \quad (26)$$

Where  $\Delta v_{st}$  represents the variation in  $v_{st}$  induced by variations in  $v'$  of  $\Delta v'$  among a group of measuring devices. With the realization that  $v'$  itself may be made relatively small by appropriate selection of short tubing of narrow bore, it is seen that  $\Delta v'$  may be made to approach an insignificantly small magnitude. Certainly there is no technical difficulty in arranging to have  $\Delta v'$  less than 1 cc.; this corresponds to about  $2\frac{1}{4}$  inches of  $\frac{3}{16}$  inch bore tubing. Further  $|T_s - T_r|$  may be given a maximum value of say  $10^\circ\text{C.}$ , and  $T_r$  a minimum value of  $290^\circ\text{K.}$  It is unlikely that  $P_s$  will exceed say 780 mm. Under these conditions of maximum dependence of  $v_{st}$  upon fluctuations in  $v'$ , we may write

$$\Delta v_{st} = 0.036 \text{ cc.}$$

as the maximum deviation in  $v_{st}$  to be expected from this source.

We may now consider the dependence of  $v_{st}$  upon fluctuations in  $V'$ .

$$\Delta v_{st} = \frac{T_{st}}{T_s} \left( \frac{P_r - P_s}{P_{st}} \right) \Delta V' \quad (27)$$

Now  $V'$  is limited in smallness by the mass of dough to which the equipment is adapted. It has been this writer's experience that  $V'$  may not be much smaller in cubic centimeters than some ten times the mass of the dough in grams. This ratio permits somewhat more than sufficient head room necessary for free expansion of the dough. Hence  $\Delta V'$  among a group of volumetric systems may possess a significant magnitude.

We may assume, however, that with ordinary precautions  $\Delta V'$  among a group of vessels may be kept below 5 cc. Further, it is unlikely that  $T_s$  would ever be smaller than 290°K, or that  $|P_r - P_s|$  would exceed 5 mm. Hence we may calculate the deviation induced in  $v_{st}$  under the worst likely conditions:

$$\Delta v_{st} = 0.034 \text{ cc.}$$

We conclude that within the limits set for  $\Delta v'$  and  $\Delta V'$  one may expect  $v_{st}$  to be within 0.07 cc. of the correct volume, independent of the total volume of gas involved. This may be compared to the effects of similar variations of  $V'$  upon the  $v_{st}$  obtained via the manometric method:

$$\Delta v_{st} = \frac{h + 5}{760} 5$$

It is apparent that the variation in  $v_{st}$  is dependent upon the reading  $h$ . Even the relatively small value of  $h = 100$  mm. induces an error in  $v_{st}$  of 0.691 cc.

Here at least the volumetric procedure possesses an advantage over the manometric. Less difficulty is involved in the construction of a group of replicate measuring systems. It may be argued, of course, that  $V'$  may be made so large that a variation in  $V'$  of 5 cc. may be considered without significance. Under these conditions, however, the size of dough must be increased proportionately if appreciable magnitudes of  $h$  are to be obtained. This involves other considerations such as the amount of gas confined within the dough mass and the difficulty in maintaining no more than, say, a 5 cc. difference in volume among a group of large vessels. A point of interest lies here. The study of gas production is basically the study of the number of moles of carbon dioxide produced; that is, the gas must be measured under conditions of known pressure and temperature. It is therefore advisable to arrange the determination so that the dough under consideration possesses as large a surface compared to its mass as is practical. This speaks for small doughs.

The smallness of the dough, however, is limited by the necessity of maintaining a dough large enough to be representative of the ingredients involved. The possibility of avoiding these difficulties by the use of suspensions rather than doughs is not to be given overly much attention, since it will be shown in a later paper of this series that gas production follows a radically different and less informative course under these conditions.

It may be well at this point to consider equipment actually in use. A manometric set-up on the market employs a metal fermentation

vessel, 261 cc. in volume. This vessel is threaded at its mouth. The cover, to which the manometer is attached, is screwed down upon a rubber gasket of some 2 mm. thickness. The error in  $V'$  due merely to difference in compression of 1 mm. is 3.97 cc. in 244 cc. (17 cc. deducted from  $V$  because of ingredients) or 1.6%. This corresponds to 1.6% error in either  $h$  or  $v_{st}$ .

We may attempt a more detailed study of this device and compare it to a similar study of the volumetric apparatus employed in this laboratory. We will assume minimum variations in  $V'$  for both types of systems of 5 cc. and minimum variations in  $v'$  for the volumetric systems of 1 cc.  $P_r$ ,  $P_s$ ,  $T_r$ , and  $T_s$  will be so chosen as to produce maximum likely errors; that is,  $P_r = 780$  mm.,  $P_s = 775$  mm.,  $T_s = 300^\circ\text{K}$ ., and  $T_r = 290^\circ\text{K}$ . wherein it is assumed that  $P_r - P_s > |5|$  mm. and  $T_s - T_r > |10^\circ|$  are unlikely possibilities. Table I presents the critical characteristics of the two types of instruments. The figures represent cubic centimeters.

TABLE I

	$V$	$v'$	$v^*$	$V'$	$\Delta v'$	$\Delta V$
Manometric	261	—	17	244	—	5
Volumetric	83	15	8	90	1	5

Ten grams of flour are suggested for use in the manometric device; five grams of flour in the volumetric. We may calculate the deviation between the uncorrected and the corrected readings obtained via both types of instruments for the case in which  $v_{st} = 100$  cc. and  $v_{st} = 50$  cc. for the manometric and the volumetric systems respectively. For the former case we find  $h_{\text{uncor.}} = 311.5$  mm. and  $h_{\text{cor.}} = 300.1$ . This corresponds to a 3.8% error in  $h$  for the manometric method. For the latter case we find  $v_{\text{uncor.}} = 45.55$  cc. and  $v_{\text{cor.}} = 50.00$  cc. This corresponds to a 8.9% error in  $v$  for the volumetric method. We may conclude that under the worst environmental and instrumental conditions, and if no corrections are attempted, the manometric equipment here under consideration will yield the more precise results.

### Discussion

The advantage of some 5% in uncorrected precision of the manometric over the volumetric technique is offset by the following considerations: (1) The problem of preventing leakage in the manometric method under conditions of some two to three hundred millimeters of mercury pressure, compared to the practically zero pressure differential in the volumetric procedure. (2) The difficulty of determining whether or not the closed manometric system is or is not

completely sealed. One would have to force air into the system in order to check for such leakage, while with the volumetric procedure it suffices merely to lower the leveling bulb and note the constancy of reading. (3) The incidental but inherent distinction between fermentation under conditions of constant volume and variable pressure as compared to fermentation under conditions of constant pressure and variable volume. The latter technique simulates actual working conditions, and therefore permits use of gas production data obtained thereby in the interpretation of the baking test and in the study of fermentation kinetics; whereas it remains to be proven that gas production under variable pressure may be considered equivalent to gas production under constant pressure. The isolated note by Sandstedt and Blish (1936) barely can be considered supporting evidence. It can be shown that effects completely masked in plots of  $v_{st}$  against time become quite marked in plots of rates of gas production against time. (4) The greater fragility of manometric equipment. (5) The greater care required in maintenance of such equipment. Purity of mercury and cleanliness of manometer are relatively important.

One additional point is worthy of consideration. In some instances rates of gas production rather than total volumes of gas produced are of interest. The average rate of gas production  $\bar{R}_{st}$  may be defined as the difference between  $v_{st2}$  and  $v_{st1}$ , where  $v_{st2}$  represents the corrected total volume of gas produced at some time indicated by the subscript 2 and  $v_{st1}$  represents the corrected total volume of gas produced at some earlier time represented by the subscript 1. This laboratory has found quarter-hour differences to be a rather close approach to corresponding instantaneous rates obtained by suitable extrapolation of one, half, quarter, and twelfth hour rates. Hence the units of  $\bar{R}$  will be considered cubic centimeters per quarter hour per 10 or 5 g. of dough respectively for the manometric and volumetric procedures described above. Equations 5 and 17 may be rewritten:

$$\bar{R}_{st} = \frac{h_2 - h_1 + P_{r2} - P_{r1}}{P_{st}} V' \quad (5'')$$

and

$$\bar{R}_{st} = \frac{T_{st}P_s}{T_sP_{st}} \left[ \frac{P_{r2} - P_{r1}}{P_s} V' + T_s \left( \frac{T_{r1} - T_{r2}}{T_{r1}T_{r2}} \right) v' + \frac{T_s}{P_s} \left( \frac{P_{r2}}{T_{r2}} v_{r2} - \frac{P_{r1}}{T_{r1}} v_{r1} \right) \right] \quad (17')$$

But it is extremely unlikely that  $|T_{r1} - T_{r2}|$  will exceed  $1^\circ\text{C.}$  during the quarter hour of time involved. Further, it may be assumed that  $|P_{r2} - P_{r1}|$  will not exceed 1 mm. during this interval. By the pro-

cedure employed in an earlier section of this paper we will calculate  $(h_2 - h_1)$  corrected and uncorrected corresponding to a given  $\bar{R}_{st}$  and will also calculate the corresponding uncorrected  $(v_{r2} - v_{r1})$  for the volumetric case.

We will again assume values for the  $T$ 's,  $P$ 's,  $v'$  and  $V'$  such that maximum deviations between corrected and uncorrected values will be produced. For this purpose we select:  $P_{r2} = 780$  mm.;  $P_{r1} = 779$  mm.;  $P_s = 775$  mm.;  $T_{r1} = 291^\circ\text{K.}$ ;  $T_{r2} = 290^\circ\text{K.}$ ;  $T_s = 300^\circ\text{K.}$ ;  $v' = 16$  cc.;  $V'$  (manometric uncorrected) = 244 cc.;  $V'$  (manometric corrected) = 249 cc.;  $V'$  (volumetric) = 95 cc.  $\bar{R}_{st}$  will be assumed 12.00 cc. and 6.00 cc. per quarter hour for the manometric and the volumetric methods respectively.  $v_{r2}$  will be assumed 40.00 cc. for the latter method. We find:  $(h_2 - h_1)$  uncorrected = 37.39 mm. and  $(h_2 - h_1)$  corrected = 35.60 mm. This corresponds to a 5.0% error in  $\bar{R}_{st}$ . For the volumetric method we find:  $(v_{r2} - v_{r1})$  uncorrected = 5.30 cc. This corresponds to an error of 11.7% in  $\bar{R}_{st}$ , if no appropriate corrections are made. Yet the method in this laboratory has been found to yield replicate results in  $\bar{R}$  with a maximum spread of no more than 2%-3%.

If one is content with this precision, then equation 17' may be simplified as follows: Consider the term

$$T_s \left( \frac{T_{r1} - T_{r2}}{T_{r1} T_{r2}} \right) v'$$

Since the maximum divergence of all temperatures ordinarily does not exceed 5%, and since the term in question is merely a corrective term of only some 0.10 cc. under our conditions, we are justified in replacing  $T_s$ , and  $T_{r1}$  and  $T_{r2}$  of the denominator by  $T_{st}$ . The term

$$\frac{T_s P_{r2}}{P_s T_{r2}} v_{r2} - \frac{T_s P_{r1}}{P_s T_{r1}} v_{r1}$$

may be replaced by

$$\frac{T_s P_r}{P_s T_r} (v_{r2} - v_{r1})$$

since  $P_{r2}$  and  $T_{r2}$  are not expected to differ from  $P_{r1}$  and  $T_{r1}$  by more than 0.2% and 0.4% respectively and we may take  $P_{r1} = P_{r2} = P_r$  and  $T_{r1} = T_{r2} = T_r$ . Equation (17') now has the form

$$\bar{R}_{st} = \frac{T_{st}}{T_s} \left( \frac{P_{r2} - P_{r1}}{P_{st}} \right) V' + \frac{P_s}{P_{st}} \left( \frac{T_{r1} - T_{r2}}{T_{st}} \right) v' + \frac{T_{st} P_r}{T_r P_{st}} \bar{R}_r \quad (17'a)$$

But

$$\frac{T_{st}}{T_s} \left( \frac{P_{r2} - P_{r1}}{P_{st}} \right) V'$$

is merely a corrective term of at most some 0.20 cc. and since the maximum likely error introduced by letting  $T_{st} = T_s$  is only 5%, the factor  $T_{st}/T_s$  may be omitted. By the same reasoning the factor  $P_s/P_{st}$  of the second term is avoided. Our simplified relationship for correcting rates of gas production to conditions of standard pressure and temperature finally takes the form:

$$\bar{R}_{st} = \frac{P_{r2} - P_{r1}}{P_{st}} V' + \frac{T_{r1} - T_{r2}}{T_{st}} v' + \frac{T_{st}P_r}{T_rP_{st}} \bar{R}_r \quad (17'b)$$

where the subscript  $r$  designates a value midway between that designated by  $r_1$  and  $r_2$ .

Within similar limitations, equation 17 appears as:

$$v_{st} = \frac{P_r - P_s}{P_{st}} V' + \frac{T_{r1} - T_{r2}}{T_{st}} v' + \frac{T_{st}P_r}{T_rP_{st}} v_r \quad (17a)$$

### Conclusions

Errors due to variations in barometric pressure, room temperature, and to variations in physical characteristics of replicate measuring systems may be of sufficient magnitude to warrant the application of corrective measures. If such corrections are not made, then the incidental data required to perform such operations should be recorded. The volumetric method is more sensitive to variations of pressure and temperature than is the manometric. The converse, however, is true for variation in the physical characteristics of replicate measuring devices. Finally, considering the manometric method, data recorded in units of pressure are lacking in physical significance unless the total internal volume available for free expansions of gas is recorded as well.

### Literature Cited

- Bailey, C. H.  
 1939 Measuring fermentation rate and gas losses in dough. *Cereal Chem.* **16**: 665-671.  
 — and Johnson, A. H.  
 1924 Carbon dioxide diffusion ratio of wheat flour doughs as a measure of fermentation period. *Cereal Chem.* **1**: 293-304.  
 Blish, M. J., Sandstedt, R. M., and Astleford, G. R.  
 1932 Sugars, diastatic activity, and "gassing power" in flour. *Cereal Chem.* **9**: 388-390.  
 Sandstedt, R. M., and Blish, M. J.  
 1934 Yeast variability and its control in flour gassing power tests. *Cereal Chem.* **11**: 370-372.  
 1936 Effect of pressure on rate of gas production in yeast fermentation. *Cereal Chem.* **13**: 789-790.



# **GAS PRODUCTION IN YEAST FERMENTATION AND ITS APPLICATIONS. II. A VOLUMETRIC METHOD FOR THE STUDY OF GAS PRODUCTION**

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(Received for publication February 2, 1940)

Many chemical phenomena in liquid solutions are characterized by the liberation of a gaseous phase. The study of both the intensity and the extent of gas production has often led to information concerning the mechanism of the reaction. It is therefore not surprising that methods for the determination of gas production have received considerable study and development by many workers in many fields. The study of nitrosotriacetone-amine by Brönsted and King (1925), of yeast fermentation by St. John and Bailey (1929), Jørgensen (1931), Blish, Sandstedt, and Astleford (1932), and by Bailey (1939), and the determination of sugars via appropriate enzymes by Schultz and Landis (1932) constitute cases in point.

The problem of designing a method for the measurement of gas formation is then more a matter of adaptation of existing procedure rather than that of origination. This writer has shown (Part I of this series) that both of the alternative principles, measurement of volume under conditions of constant pressure, or measurement of pressure under conditions of constant volume, suffer relative advantages and disadvantages. Certain conditions were laid down for minimizing disturbing influences, such as fluctuations in temperatures and atmospheric pressure. Incidental advantages were ascribed to the volumetric technique, such as relative simplicity and strength of equipment, ease of maintaining essential cleanliness, and lower risk of leakage. A direct advantage lay in its measurement of gas production under conditions of constant pressure and the associated elimination of pressure as a variable—important or unimportant as it may prove to be. It is the purpose of this paper to describe the apparatus and the procedure which have in our hands during the past three years yielded data of relatively high precision concerning gas production in yeast-fermenting doughs.

## **Apparatus**

Since in the procedure hereinafter to be discussed, 85.0 g. of dry flour (100.0 g. on a 15% moisture basis) are used, it is convenient to have a set of masses calibrated in 0.5% units of moisture such that a given mass represents that mass of flour of corresponding moisture content which is equivalent to 85.0 g. of said dry flour. The flour is

to be weighed into counterpoised pint Mason jars, the jars covered, and permitted to rest in the constant-temperature baking room until room temperature is reached; that is, the flour as well as all other ingredients is brought to  $28^{\circ}\pm 1^{\circ}\text{C}$ . before mixing, thereby avoiding temperature variability in the mixed dough. Balances used in weighing are such that all ingredients may be weighed to within 0.2% of the desired value. Water is delivered from a 100-ml. burette into 100-ml. Erlenmyer flasks, the flasks stoppered and stored until used. Yeast is weighed into Coors No. 000 evaporating dishes, covered with watch glasses, and also stored until used. This generally is at most an hour and a half. Storage for this period of time has, so far as we have been able to detect, no effect upon the subsequent behavior of the yeast. This agrees with the findings of Merritt, Blish, and Sandstedt (1932), with those of Cook and Malloch (1930), and with those of Sandstedt and Blish (1934). Mixing is carried out in a two-speed, double-unit Washburn Crosby mixer. Slow corresponds to 110 rpm. and fast to 220 rpm. Each unit possesses a hook mixing arm. The measuring systems and the fermentation chambers are described beneath their respective figures.

### Procedure

All ingredients are weighed out in advance of mixing. One-hundred-gram samples of flour (15% moisture basis) are weighed directly into the Mason jars; 3.00-g. samples of yeast into the evaporating dishes; and distilled water to make 60% absorption (15% moisture basis) delivered from the 100-ml. burette into the Erlenmyer flasks. Supplementary ingredients are weighed on an analytical balance and, if soluble, are dissolved into their respective waters; if insoluble they are placed in individual evaporating dishes and incorporated into the dough in a fashion similar to that of the yeast. The "primitive formula" implied above was suggested to this writer some time ago by E. E. Werner.

The flours for runs No. 1 and No. 2 are placed each in one of the mixing bowls. On the quarter hour, Erlenmyer flask and evaporating dish No. 1 are uncovered. Some 10 ml. of the water is poured over the yeast. The mixture is stirred vigorously by means of a heavy pestle-like glass rod and transferred to mixing bowl No. 1. The dish is washed clear by three additional rinsings, after which the remaining liquid is poured into bowl No. 1. These operations should require one minute. Ingredients No. 2 are added to bowl No. 2 during the second minute prior to mixing. Mixing is begun two minutes after the quarter hour and consists of one minute on low and one minute on high. The dough temperature out of the mixer should be within  $1^{\circ}$  of  $30^{\circ}\text{C}$ ., and the time is four minutes past the quarter hour.

Two 8.00-g. aliquots are taken from each dough and transferred to their respective vessels *A* (Fig. 2). These are attached to the measuring systems by means of the clamps *F* (Fig. 2) so that the aliquots from dough No. 1 go to burettes 1 and 2, while those from dough No. 2 go

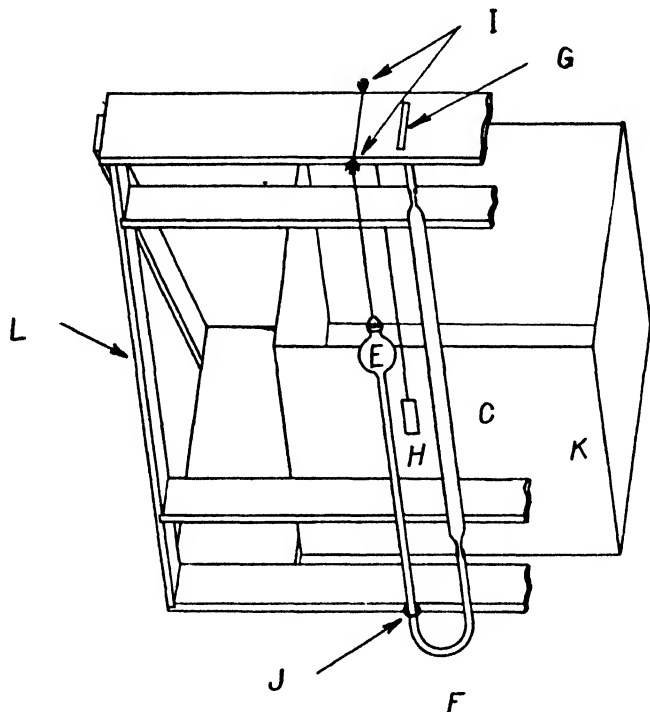


Fig. 1. The measuring system. Ten 100-ml. Hempel gas burettes, one of which, *C*, is illustrated above, are mounted on the wooden framework, *L*. Their lower outlets are connected via  $\frac{1}{4}$ -inch Neoprene tubing through eyes, *J*, to 100-ml. leveling bulbs, *E*. Tubing, burettes, and leveling bulbs are filled with paraffin oil. The latter are supported by braided strings which ride the pulleys, *I*, and are counterbalanced by the weights, *H*. The upper outlets of the burettes are connected respectively to the T-tubes, *G*, the front openings of which are subject to sealing and the rear openings of which are attached to the tubing connectors, *D*, of the fermentation chambers (Fig. 2) by means of  $\frac{1}{4}$ -inch Neoprene tubing. Total internal volume,  $v'$ , of the connections from top of *C* to *D* is some 14 ml. and is a constant common to the ten units. *K* is a constant-temperature water bath in which the fermentation chambers are immersed. It is equipped with an electrical stirrer, a 500-watt Cenco Knife heater, and a toluene-mercury thermoregulator. A thermionic relay avoids fouling at the mercury-platinum contact. Temperature is maintained at  $30 \pm 0.05^\circ\text{C}$ . Lastly a brass framework is mounted within the bath. This supports the fermentation chambers by means of their extended brass plugs, *C* (Fig. 2).

to burettes 3 and 4. Precisely 13 minutes after the start of mixing (on the following quarter hour) system 1 is sealed by clamping the tubing attached to the front outlet of the T-tube, *G* (Fig. 1). Thirty seconds later, system 2 is sealed; similarly in half-minute intervals systems 3 and 4. Immediately after system 4 is sealed, ingredients No. 3 and No. 4 are transferred to their mixing bowls. Transferring should be completed and mixing begun on the nineteenth minute after the initial quarter hour. The aliquots for doughs No. 3 and No. 4 go to measuring systems 5, 6, 7, 8. Burette 1 is read to the nearest 0.05 ml. on the second quarter hour after mixing and in successive half-minute steps burettes 2, 3, and 4 are read, and burettes 5, 6, 7, and 8 are sealed.

Immediately thereafter the ingredients for the fifth dough are transferred to the mixer, and two minutes after the reading of burette 8, the last mix is started. And so on in half-minute steps first sealing,

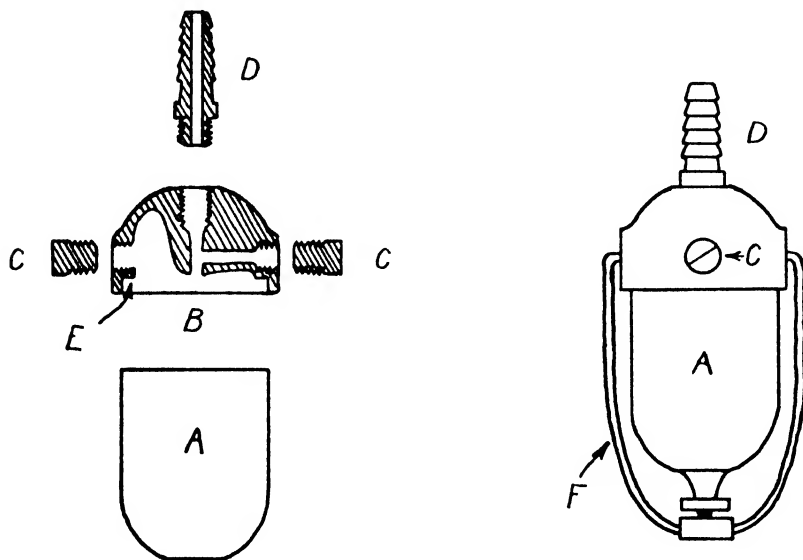


Fig. 2. Details of fermentation chamber: left, front cross section; right, side section view. These consist of reconstructed Tillotson Model OW-400A fuel strainers. *B* represents a cross section of the head. *E* is an inset ridge which serves as backing for a rubber gasket of 4-mm. thickness. The cork gasket supplied with the filter is unsatisfactory. *F* is a clamp which permits one to force the glass vessel *A* into close contact with the gasket. The internal volume of the fermentation chamber, *V*, is about 83 ml. The total internal volume from burette to and including chamber is then 97 ml. And since we will consistently employ an 8.00-g. aliquot of dough of volume  $v^*$ , assumed 8 ml., the total free volume of the system defined by  $V' = V + v' - v^*$  is equal to 89 ml.

then subsequent quarter hourly readings of each of the measuring systems are made. Hence, the volume of gas produced as a function of time is obtained.

### Significance of Rate of Gas Production<sup>1</sup>

It may prove worth while to go into somewhat greater detail concerning the definition and physical significance of the rate of gas production. More precisely, it should be called the time rate of gas production. By this is meant the volume of gas produced in a given

<sup>1</sup> The cereal literature often speaks of "gas production," "rate of gas production," "gas retention," and "gassing power." Unfortunately, these terms have been used somewhat loosely, and the word "rate" has often proxied for "volume" and both have been supplanted on occasion by "power." The unit of volume is generally the cubic centimeter, or perhaps better, the milliliter. We therefore define "gas production" as the number of milliliters of carbon dioxide, corrected to standard temperature (30°C.) and pressure (760 mm. of Hg) that is produced from time of mixing to time of reading under standard conditions of fermentation. The use of "gassing power" is neither quite correct in implied units, nor quite fortunate in its visual similarity to horsepower. Properly, were one interested in measuring power of fermentation, one would determine the rate of gas production say in milliliters per minute and multiply this result by the atmospheric pressure say in dynes per square centimeter. It remains, therefore, that rate rather than volume of gas produced is a measure of power, though even this is not power itself. "Gassing power" seems like a pretender to the position of flour characteristic. It may appear that these distinctions are somewhat academic in the present instance and that the matter of constant factors may well be neglected. One point, however, remains as a significant detail. That which usually is determined as "gassing power" is an average rate and hence a measure of an average power. "Gas retention" apparently implies the maximum volume attainable by a dough under fixed conditions of fermentation. See St. John and Bailey (1929), Working (1929), and Bailey (1939). More will be said about this at another time.

unit of time. For practically all chemical and physical phenomena this function varies continuously with time; that is, if it is plotted against time it yields a smooth curve without discontinuities. Mathematically, it may be represented by the change in volume of gas produced divided by the change in time from one measurement of volume to the other. Symbolically it is represented by

$$\bar{R} = \frac{\Delta v}{\Delta t}. \quad (1)$$

Here  $\bar{R}$  represents the average rate of gas production for the time interval  $\Delta t$ .  $\Delta v$  represents the change in volume during the interval  $\Delta t$ . If the interval of time between the two measurements of volume is decreased till it approaches zero, then the infinitesimal amount of gas produced divided by the infinitesimal period of time yields the instantaneous rate of gas production at a time lying somewhere within the infinitesimal time interval of measurement. That is, the instantaneous time rate of gas production is associated with and is a function of a perfectly defined instant of time. Symbolically this may be represented by

$$R = \frac{dv}{dt}. \quad (2)$$

It is this quantity,  $R$ , that possesses mathematical significance but which unfortunately is not yet subject to direct measurement.<sup>2</sup> It can, however, be approached by  $\bar{R}$ , the average time rate of gas production.  $R$  is subject to far simpler theoretical treatment than  $v$ , and once obtained as a function of time can be converted to  $v$  by ordinary mathematical techniques of integration. Once  $v$  is obtained as a function of time, obviously  $\bar{R}$  is obtained as well. The significance of  $R$  may be represented simply by the following expression

$$R = \frac{dv}{dt} = KZ_t G_t + K'Z'_t F_t, \quad (3)$$

where  $K$  is the so-called velocity constant for glucose fermentation,  
 $K'$  is the so-called velocity constant for fructose fermentation,  
 $Z_t$  is the concentration of zymase effective in glucose fermentation at time  $t$ ,  
 $Z'_t$  is the concentration of zymase effective in fructose fermentation at time  $t$ ,  
 $G_t$  is the concentration of glucose at time  $t$ , and  
 $F_t$  is the concentration of fructose at time  $t$ .

<sup>2</sup> Note the ingenious attempt by James and Huber, *Cereal Chem.* 5: 181-182 (1928). Water displaced by gas production enters a cylinder at the base of which is a small orifice. The rate of gas production (rate of inflow of water),  $R$ , is measured by the height of water,  $h$ , in the cylinder. It is evident that  $dh/dt = C(R - r)$  where  $C$  is the ratio of  $h$  to volume of water in cylinder and  $r$  represents the rate of leakage through orifice. But  $r = f(h)$ . Therefore  $R = 1/C \, dh/dt + f(h)$  and is not measured by  $h$  itself. An uncertainty of order  $1/C \, dh/dt$  is involved. This is equivalent in practice to the averaging of  $R$  over a finite time interval,  $\Delta t$ .

It is understood that temperature and pressure are held constant. Also implicitly, it is assumed that either only hexoses are fermentable or that the higher saccharides, by whatever mechanism they may, must pass through the hexose stage. Nothing further can be done with this until  $Z_t$ ,  $Z_t'$ ,  $G_t$  and  $F_t$  are evaluated as functions of time. The problem is not necessarily impossible of solution and if solved would permit of definite understanding of the effects of those ingredients which influence the rate of gas production.

It has been suggested that the instantaneous time rate of gas production is not subject to direct measurement but that it may be approached as closely as we please (within the limits of our instruments) by making the time intervals between measurements as small as possible, or at least so small that further decreases in the time interval do not affect the results obtained. Therefore as is evident from our procedure, the difference in volume between any two consecutive readings of a given burette may be considered the average rate of gas production in milliliters per quarter hour per 8 g. of dough at the average time lying midway between the two actual times at which the necessary readings were made.

Our practice, when plotting rates versus time, has been to associate a given rate with the second of the two times involved. This simplifies recording and plotting of data, and if done consistently in no manner affects the relative interpretation of the plots; it is equivalent to shifting the entire curve  $7\frac{1}{2}$  minutes in the direction of greater time. Hence if one wishes to compare rates obtained with  $\Delta t$  of say 5, 15, and 45 minutes, he must be certain to select average rates obtained at corresponding average times. For example, consider Tables I and II, in which typical data are recorded, readings having been taken at five-minute intervals rather than at our usual quarter-hour intervals. These rates, corrected to similar units, are plotted against their corresponding average times in Figure 3. We wish to indicate that the difference between the five-minute and the quarter-hour rate curves is no greater than the experimental error involved in the determination of the five-minute rates, and we are therefore justified in at least tentatively considering quarter-hour rates as a satisfactory approach to instantaneous rates. Hence, through such measurements, we may attempt to study the functional relationship, equation 3. Further, a glance at Tables I and II or study of the procedure used in obtaining these data, is sufficient to indicate a certain lack of absolute definition in the burette readings of volume; that is, there is an unknown and very likely variable quantity of gas formed during the first quarter-hour. Its magnitude may be established by extra-polation of either the volume or rate-versus-time curve to zero time. Mere observation

TABLE I

AVERAGE RATE OF GAS PRODUCTION ( $\Delta t = 5$  MINUTES) AS A FUNCTION OF TIME

Minutes <sup>1</sup>	Volume (ml.)	5-min. rate <sup>2</sup> (ml./5')	5-min. rate <sup>3</sup> (ml./15')
0	Mixing begun	—	—
5	—	—	—
10	—	—	—
15	0.00	System sealed	—
20	0.95	0.95	—
25	2.00	1.05 (22½')	3.15
30	3.10	1.10	—
35	4.45	1.35	—
40	5.95	1.50 (37½')	4.50
45	7.55	1.60	—
50	9.15	1.60	—
55	10.80	1.65 (52½')	4.95
60	12.40	1.60	—
65	13.90	1.50	—
70	15.35	1.45 (67½')	4.35
75	16.70	1.35	—
80	18.10	1.40	—
85	19.45	1.35 (82½')	4.05
90	20.85	1.40	—
95	22.25	1.40	—
100	23.80	1.55 (97½')	4.65
105	25.45	1.65	—

<sup>1</sup> Time from start of mixing, the average one minute shifted, mentioned at end of section on consistent errors, being neglected.

<sup>2</sup> Figures in parenthesis are the average times of which the average rates are a function.

<sup>3</sup> Rates of preceding column multiplied by three.

TABLE II

AVERAGE RATES OF GAS PRODUCTION ( $\Delta t = 15$  AND 45 MINUTES RESPECTIVELY) AS FUNCTIONS OF TIME

Minutes	15-min. rate (ml./15')	45-min. rate (ml./45')	45-min. rate <sup>1</sup> (ml./15')
0	Mixing begun	—	—
15	System sealed	—	—
30	3.10 (22½')	—	—
45	4.45 (37½')	—	—
60	4.85 (52½')	12.40 (37½')	4.15
75	4.30 (67½')	13.60 (52½')	4.55
90	4.15 (82½')	13.30 (67½')	4.45
105	4.60 (97½')	13.05 (82½')	4.35

<sup>1</sup> Rates of preceding column divided by three.

of such graphs will show the relatively higher precision of rate extrapolations. This is at least one advantage in using rate- rather than volume-versus-time curves as a means of recording data, both for one's own use and for publication purposes. A second overwhelming advantage may also be mentioned at this time. The rate-versus-time curve is roughly some ten times as compact; that is, for a given amount of available space one may plot to an additional significant figure.

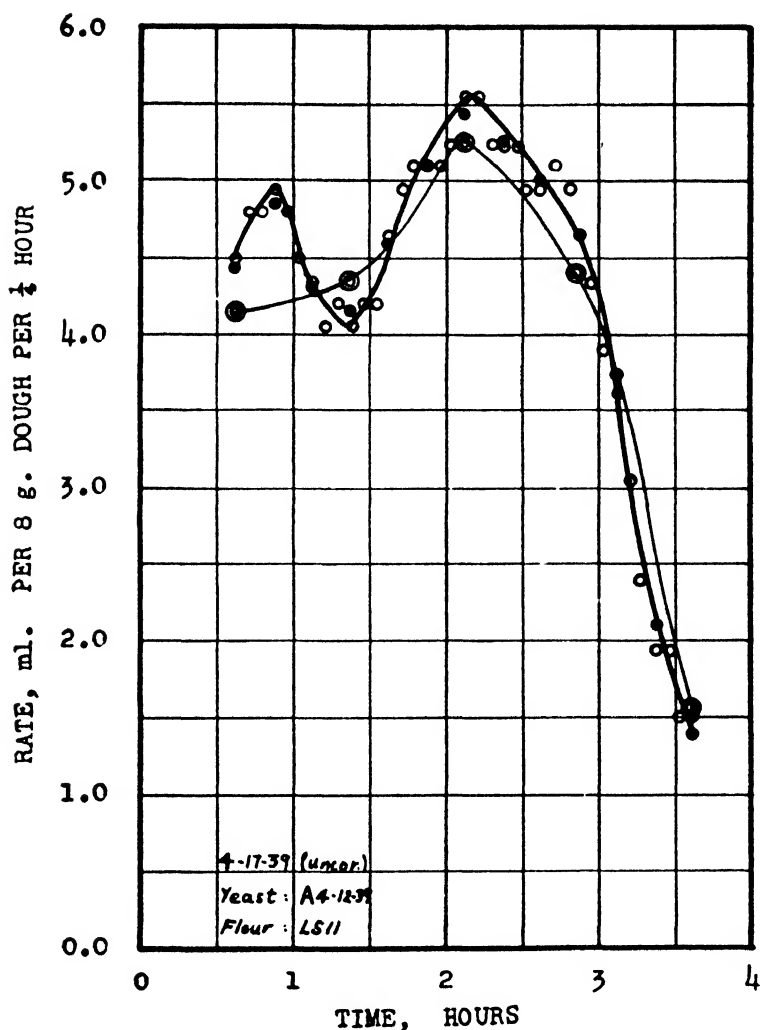


Fig. 3. Variation of average rate of gas production with variation of interval between measurements of volume. Open circles, 5-minute intervals; closed circles, 15-minute intervals; double circles, 45-minute intervals.

### Consistent Errors

At the risk of appearing somewhat overcritical, we may now consider a source of barely tangible consistent error introduced by the procedure. There is a degree of uncertainty concerning the zero time—that is, concerning the instant at which fermentation starts. We may arbitrarily consider this time to be synonymous with the moment mixing begins.

By so doing, burette 1 is sealed 13 minutes after, while burette 4 is sealed 14½ minutes after the start of fermentation. Hence, an error due to this 1½-minute shift along the time axis may be involved when results obtained via random measuring systems are indiscriminately compared. The magnitude of this error is not sufficiently large, at



least for our present purposes, to warrant corrective measures in procedure. Its order, however, may be estimated as follows: The maximum rate of gas production as observed under our conditions is some 6.5 ml. per quarter, per 8 g. of dough. Thus, since the maximum error in total volume of gas produced occurs at the time of maximum rate, this error must be 0.65 ml. The volume of gas formed up to this time is generally some 30 ml. Therefore the maximum percentage error in volume from this source may be placed at about 2%.

In practice, however, one is not usually interested in the volume of gas produced from zero time to time of maximum rate; rather, one is interested in the total volume of gas produced up to the time at which the yeast becomes relatively exhausted. At this time, the rate of gas production is about 1 ml. per quarter hour and the total volume of gas produced is some 50 mls. Hence, the  $1\frac{1}{2}$ -minute shift in time results in only a 0.2% error in volume.

One may likewise consider the error introduced in the measurement of the rate of gas production. The controlling factor is the acceleration—or change in rate. Normally this is not greater than 2 ml. in absolute magnitude per quarter hour per quarter hour. At the time of maximum acceleration the rate generally lies between 3 and 5 ml. per quarter hour. Therefore the maximum error in the rate of gas production due to the source under consideration may not be expected to exceed 0.20 ml. per quarter hour, or some 4%–7%. As is evident this magnitude of error occurs only at inflection points on the rate-versus-time relationship. The error is practically nil at maxima and at minima. Furthermore, the additional half-minute shift along the time axis involved in assuming that the time of sealing follows mixing by a quarter hour rather than by  $14\frac{1}{2}$  minutes may be neglected. We may, however, with an average error in time no greater than one minute assume, if the need arises, that all burettes are sealed on the average 14 minutes after their respective starts of mixing; that is, we can therefore cut the maximum time shift from 2 minutes to 1 minute.

### General Discussion

The first of the essential requirements to be fulfilled by measurements of any kind is that of reproducibility. Figure 4 represents a group of seven individual determinations of rate as a function of time upon the same ingredients under identical conditions, and upon the same day. The width of the curve, or band, indicates the maximum spread among measurements, and denotes the precision of which the measuring system and procedure are capable. The average volume of gas produced in  $3\frac{3}{4}$  hours was  $54.60 \pm 0.26$  ml. and the maximum deviation of any one determination of volume from this average was

0.45 ml. The average percentage deviation from the average is then 0.48%. This does not compare unfavorably to many analytical procedures. The average value of the rate of gas production at the second

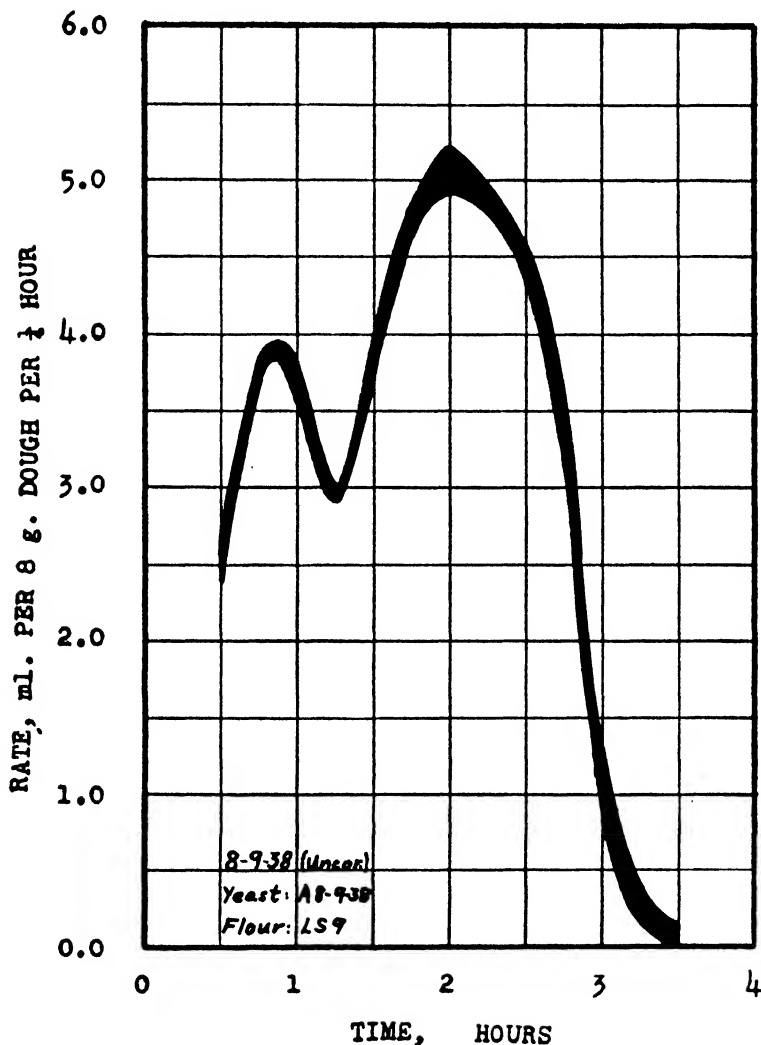


Fig. 4. Seven replicate determinations of rate of gas production as a function of time. All points are displaced downward 1.0 ml.

maximum is  $5.98 \pm 0.08$  ml. per quarter hour, and the greatest deviation from this value was 0.22 ml. in one case and 0.08 ml. or less in all others as can be seen from Table III. This corresponds to an average percentage deviation from the average of some 1.3%, which is about as good as can be expected with the present measuring systems, since the burettes are calibrated in 0.2-ml. units and estimations to 0.05 ml. cannot be considered certain.

TABLE III

SEVEN REPLICATE DETERMINATIONS OF RATE AT SECOND MAXIMUM AND 3½-HOUR GAS PRODUCTION AS INDICATION OF REPRODUCIBILITY OF METHOD

Rate at 2nd. peak (ml./15')	Deviation from average	Volume at 3½ hrs. (ml.)	Deviation from average
6.20	0.22	54.70	0.10
6.05	0.07	54.30	0.30
5.90	0.08	54.75	0.15
5.95	0.03	54.20	0.40
5.90	0.08	54.40	0.20
5.90	0.08	55.05	0.45
5.95	0.03	54.80	0.20
Average 5.98	±0.08	54.60	±0.26
or —	±1.3%	—	±0.48%

It is common practice in analytical methods, particularly when concerning reagents of questionable purity, to lay down certain specifications for such reagents, and since the procedure as outlined above possesses at least certain characteristics in common with analytical procedures, it is well to attempt some sort of standardization of the essential reagents in this case as well. Three basic reagents are involved: flour, water, and yeast.

It is obvious that for the study of yeast a standardized or standard flour is required, while for the study of flour a standardized or standard yeast is necessary. Unfortunately standardized ingredients of this type are not yet available. A standard flour, however, may be considered any flour which does not suffer changes in its rate of gas-production characteristics with period of storage. We find that flour stored at 2°–4°C. in sealed cans appears to fulfill this requirement for a period of more than a year. This cannot be definitely ascertained, however, since our only means of checking rate characteristics depends upon uniformity of yeast. Random variations from time to time in rate data of a standard flour stored under these conditions are taken by us to indicate yeast rather than flour variability. If this is true, as it appears to be, it seems possible that a group such as the cereal chemists might make some attempt to set certain rate specifications for a standard flour. Such a flour could be prepared by suitable blending of other flours, and could be packed and stored and sold just as are other analytical reagents.

Yeast is a somewhat more difficult problem. In Table IV are presented random data obtained with our standard flour over a period of some eight months. Several comments are in order. Firstly, there appears to be no consistent trend of data with time. Secondly, yeast is subject to variations in at least three major respects, maltase content, invertase content, and zymase content—these terms being used in a

TABLE IV

RATES AT MAXIMA AND FOUR-HOUR GAS PRODUCTION CORRECTED TO 30°C. AND 760 MM. OF HG OBTAINED OVER EIGHT-MONTHS PERIOD VIA FLOUR STORED IN SEALED CANS AT 2°-4°C. AND RANDOM SAMPLES OF YEAST A

Date	Rate at 1st. peak (ml./15')		Rate at 2nd. peak (ml./15')		Volume, 4 hrs. (ml.)	
12/18/39	5.00	0.00	5.40	0.12	58.40	0.72
12/13/39	4.70	0.30	5.30	0.22	58.45	0.77
11/30/39	5.05	0.05	5.50	0.02	59.55	1.87
11/27/39	5.15	0.15	5.50	0.02	57.80	0.12
11/16/39	4.90	0.10	5.60	0.08	57.80	0.12
11/15/39	4.70	0.30	5.50	0.02	57.20	0.48
11/10/39	5.50	0.50	5.85	0.33	62.15	4.47
10/30/39	5.10	0.10	5.70	0.18	58.40	0.32
10/17/39	5.90	0.10	5.35	0.17	57.65	0.03
10/ 5/39	4.85	0.15	5.40	0.12	55.00	2.68
10/ 2/39	5.00	0.00	5.50	0.02	55.60	2.08
9/29/39	4.80	0.20	5.15	0.37	54.80	2.88
9/27/39	5.15	0.15	5.40	0.12	55.20	2.48
8/28/39	5.10	0.10	5.55	0.03	56.40	1.28
7/21/39	4.90	0.10	5.55	0.03	56.00	1.68
6/26/39	5.10	0.10	5.70	0.18	55.95	1.73
6/ 6/39	4.95	0.05	5.65	0.13	57.70	0.02
5/ 5/39	5.10	0.10	5.80	0.28	59.65	1.97
4/13/39	5.15	0.15	5.55	0.03	60.45	2.77
4/11/39	4.80	0.20	5.40	0.12	59.35	1.67
Average	5.00	—	5.52	—	57.68	—
Average deviation from average	—	0.15	—	0.13	—	1.51
Percentage deviation	—	3.0%	—	2.4%	—	2.6%

sense broad enough to include any associated co-enzymes and the like. Other variable characteristics of yeast such as permeability of cell membranes undoubtedly play a role. Hence an indefinite number, certainly greater than three, of points on the rate-versus-time curve is needed to characterize the yeast; in fact a number of points sufficient to solve the unknown functional relationship between rate and time for all its parameters is required. Certain points on the curve are subject to smaller errors in measurement and correspond to a simplified form of the unknown functional relationship. These points occur at maxima and at minima. An additional point may be taken as the total volume of gas produced up to some arbitrary time at which the rate becomes so low that further increments of volume may be considered completely a flour rather than a yeast characteristic. Hence in Table IV we tentatively report rates at the first and second maxima, and volume of carbon dioxide produced in four hours. In doing so, we make the rather crude hypothesis that any two yeasts operating on a standard flour and yielding rates-of-gas-production-versus-time data such that the maxima and the four-hour gas production of the

one yeast correspond to those of the other, then the complete curve of the one is congruent with that of the other. Thirdly, the average percentage deviation from the average of rates at second maxima for data obtained over an eight-month period (Table IV) with different yeast samples of the same brand is some twice as great as the corresponding percentage deviation for data obtained on the same day with a given yeast sample (Table III). Further, the average percentage deviation from the average of four-hour volumes under the former conditions is some five times as great as for the latter conditions. Fourthly, it is our experience that variations in yeast of the order indicated in Table IV are sufficient to influence results obtained with the A.A.C.C. baking test. Fifthly, these findings concerning yeast variability are in agreement with those of Cook and Malloch (1930) but are in disagreement with those of Sandstedt and Blish (1934) and with those of Bohn and Favor (1939).

The problem of yeast standardization thus resolves itself into several distinct problems. Figure 5 serves to classify three different brands of yeast. It is to be noted that yeasts *A* and *C* both exhibit double maxima while yeast *B* displays only a tendency in this direction. The existence of two maxima under certain conditions has been reported by Lamour and Bergsteinsson (1936). They present evidence to prove that the two peaks merge into one under conditions of either sucrose excess or sucrose absence. Differences in magnitude and position of peaks obviously are related to difference in yeast characteristics. These differences may be associated with a great many causes by those interested in hypothesizing. Our own attempts we will reserve for a while longer. In reference to the three types of yeast here designated as *A*, *B* and *C*, it is our understanding that the differences indicated by Figure 5 are more likely attributable to conditions of growth and propagation rather than to any inherent differences of strain. If this is true, we may conclude that the manufacturers of yeast, though not having succeeded in producing an ingredient of reagent-like uniformity, have nonetheless performed a remarkable feat. The three types *A*, *B* and *C* show variations from time to time of order similar to that suggested by Table IV.

All data reported in this paper and all data to be presented in the future unless otherwise restricted, will be obtained with yeast of type *A*. Our selection of yeast *A* is in no manner associated with commercial utility; rather it is associated with somewhat greater general availability; and, more important, the magnitude and position of its peaks permit of sharper differentiation of flour characteristics.

The first part of the problem of yeast standardization is therefore the selection of type. Further, it is insufficient merely to typify the

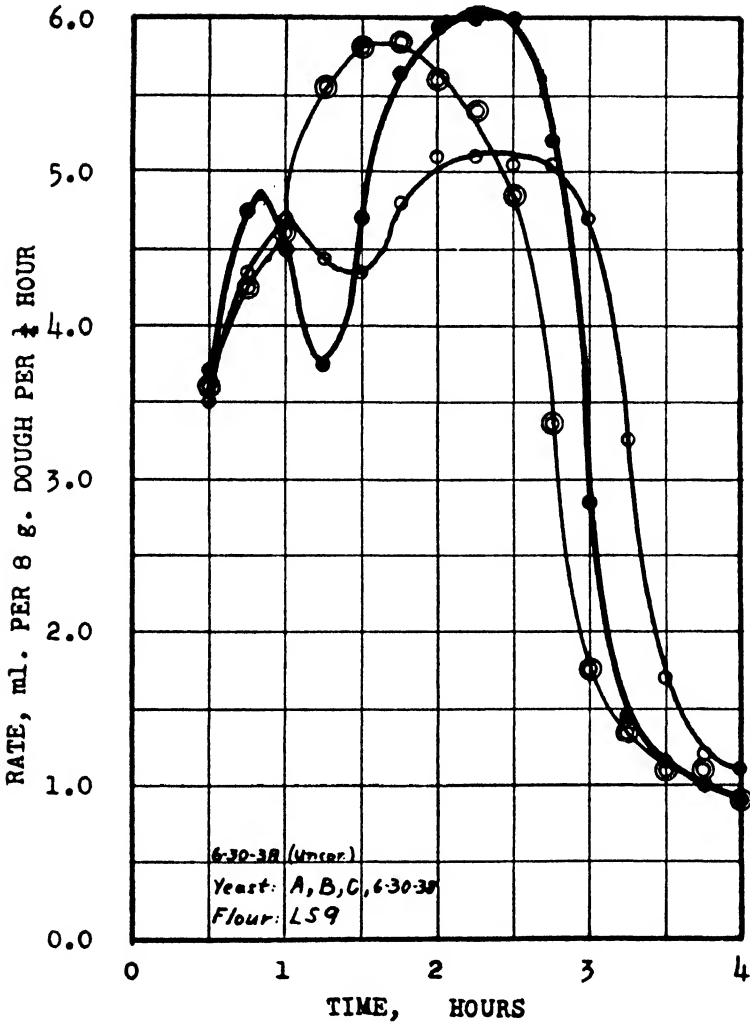


Fig. 5. Closed circles, yeast A; double circles, yeast B; open circles, yeast C.

yeast as most workers have done and are doing as A, B, C, X or Y unless characteristics such as rate-versus-time data obtained under standard conditions are recorded as well. A few isolated workers have taken the liberty of referring to yeast by brand name; others have very likely been hesitant to do so. It is evident as suggested by Eva, Geddes, and Frisell (1937) and by Bohn and Favor (1939) that a step such as here indicated must be taken before any agreement of data obtained by different workers may be expected. This writer is aware of the fact that the differences in type suggested by Figure 4 are reduced perhaps sometimes even to the vanishing point when the nutrient medium (the dough) is cluttered up with large quantities of saccharogenic material. But the differences in type, incipient or otherwise, nevertheless remain and in work of a scientific nature must

be indicated just as we unhesitatingly say phenylhydrazine by Eastman, or synthetic rubber tubing by Dupont.

Once the type is selected, the second part of the problem, the maintenance of uniformity within the type, must be approached. This unfortunately has not been done successfully as yet so far as we know. That it can be done we are certain—and in a practical fashion. One attack is at present under investigation in this laboratory. It

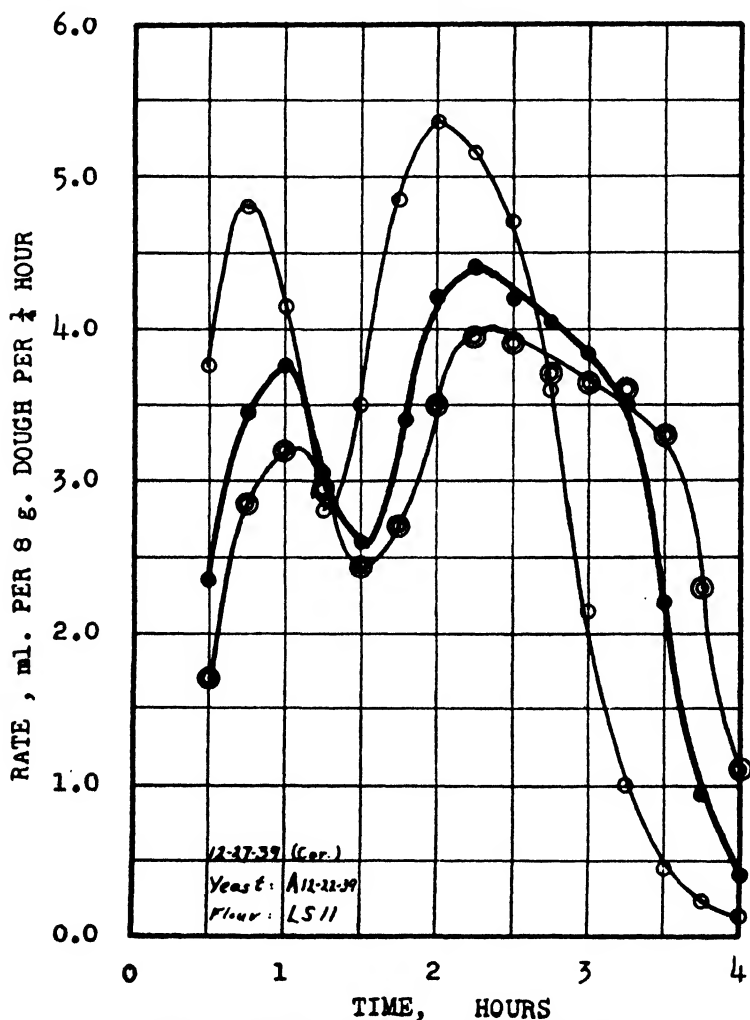


Fig. 6. Effect of variations in percentage of yeast A. All points are displaced downward 1.0 ml. Double circles, 2.6%; closed circles, 3.0%; open circles, 4.2%.

is based on the hypothesis that the ratio of the active constituents of random samples of yeast of given type is more constant than the actual quantity of these constituents. It is based further on the fact that any of the yeasts under consideration can be stored at some 4°C. for at least ten days without tangible changes in rate characteristics. We are

therefore studying the effects of variation in percentage yeast upon first and second maxima, and upon the volume of gas produced in four hours. This work is being done with our standard flour. Figure 6 represents the rate, corrected to standard temperature and pressure, as a function of time for 2.6%, 3.0%, and 4.2% yeast. The volumes of gas produced in four hours are 59.45, 60.55, 61.15 ml. respectively. These present the difficulty of refuting to some extent our hypothesis; that is, the variation in four-hour volume as indicated in Table IV cannot be attributed to only variation in effective yeast concentration, but must be attributed rather to the possible random presence of sugar in the yeast, variable glycogen storage capacity (see Sandstedt and Blish, 1939), possible variations in ratio of active constituents, or to an experimental error greater than we think likely. At any rate, our hypothesis may still hold sufficiently to permit standardization of yeast as to peak characteristics.

Table V presents the critical data. These may be described within

TABLE V

EXPERIMENTAL AND CALCULATED RATES AT MAXIMA (ML./15') AND FOUR-HOUR GAS PRODUCTION (ML.) CORRECTED TO 30°C., AND 760 MM. OF HG AS FUNCTIONS OF YEAST CONCENTRATION

Y	M <sub>1</sub> (exp.)	M <sub>1</sub> (calc.)	M <sub>2</sub> (exp.)	M <sub>2</sub> (calc.)	Volume (4 hrs.)
2.60	4.20	4.34	4.95	5.05	59.45
2.70	4.40	4.46	5.10	5.13	59.80
2.85	4.70	4.57	5.30	5.25	60.20
3.00	4.80	4.70	5.40	5.37	60.55
3.30	4.95	4.97	5.60	5.62	60.05
3.80	5.30	5.42	6.05	6.03	60.85
4.20	5.80	5.78	6.35	6.37	61.15

the range of yeast variation under consideration by the following relationships:

$$M_1 = 0.900 Y + 2.00, \quad (4)$$

$$M_2 = 0.825 Y + 2.90, \quad (5)$$

where  $M_1$  and  $M_2$  respectively refer to the rates at first and second maxima.  $Y$  represents the percentage of yeast based on flour. With these relationships established once and for all for a given standard flour, we propose to accept  $M_1 = 5.00$  and  $M_2 = 5.50$  as yeast specifications for the standardization of yeast as determined in our standard flour.

We further propose to obtain a week's supply of yeast say each Friday. On Friday or Saturday we will determine the rate of gas production for this yeast as a function of time in our standard flour and in the manner described in the procedure. We will then insert the



values of the first and second maxima into equations 4 and 5 respectively and calculate the corresponding  $\bar{Y}$  for both. The average of these figures,  $\bar{Y}$ , will then be taken as measuring the effective concentration of the 3% of yeast actually used. That is

$$\bar{Y} = 0.555 M_1 + 0.606 M_2 - 2.87. \quad (6)$$

We will then compute the quantity of yeast necessary to satisfy our specifications and will employ this quantity of the yeast in all the following week's work—and so on.

For the yeast used in obtaining the data in Table V, 3.24% is required to meet specifications. Thus for future samples of yeast, we will employ 3.00% on the preliminary standardization test and will by means of equation 6 compute the average effective concentration  $\bar{Y}$  corresponding to the observed peaks. Then by means of

$$Y' = \frac{(3.24)(3.00)}{\bar{Y}} \quad (7)$$

we will calculate  $Y'$  as the percentage of the new yeast to be used in order to maintain specifications. The results of this study will be reported upon compilation of sufficient data. The attack described is presented at this time merely as an example of what may at least be attempted in the standardization of yeast.

The third reagent required in the procedure is water. It seems self-evident to this writer that there is little choice other than the selection of distilled water.

### Summary

A refined volumetric method for the determination of gas production and a practical procedure to be used in such measurements are described in detail. Certain sources of error in these measurements and possible corrective measures are described. A tentative attack on the problem of yeast standardization is offered.

### Literature Cited

- Bailey, C. H.  
1939 Measuring fermentation rate and gas losses in dough. *Cereal Chem.* **16**: 665-671.
- Blish, M. J., Sandstedt, R. M., and Astleford, G. R.  
1932 Sugars, diastatic activity and "gassing power" in flour. *Cereal Chem.* **9**: 388-390.
- Bohn, R. T., and Favor, H. H.  
1939 Evaluation of yeast activity by means of the Sandstedt-Blish pressure meter. *Cereal Chem.* **16**: 248.
- Brönsted, J. N., and King, C. V.  
1925 Secondary kinetic salt effect in the case of hydroxyl-ion catalysis. *J. Am. Chem. Soc.* **47**: 2526-2527.
- † Cook, W. H., and Malloch, J. G.  
1930 Yeast testing. *Cereal Chem.* **7**: 137-138.

- Eisenberg, S.  
1940 Gas production in yeast fermentation and its applications. I. Volumetric vs. manometric methods for the determination of gas production. *Cereal Chem.* **17**: 417-429.
- Eva, W. J., Geddes, W. F., and Frisell, B.  
1937 A comparative study of various methods of measuring flour gassing power. *Cereal Chem.* **14**: 458-480.
- Jørgensen, H.  
1931 On the separation of "gassing power" (diastatic activity) from "strength" in baking. *Cereal Chem.* **8**: 363-366.
- Lamour, R. K., and Bergsteinsson, H. N.  
1936 Studies on experimental baking tests. III. The effect of various salts on gas-production. *Cereal Chem.* **13**: 417-418.
- Merritt, P. P., Blish, M. J., and Sandstedt, R. M.  
1932 Report of activities of the A.A.C.C. baking research fellowship. *Cereal Chem.* **9**: 220-221.
- Sandstedt, R. M., and Blish, M. J.  
1934 Yeast variability and its control in flour gassing power tests. *Cereal Chem.* **11**: 373-374.
- Schultz, A. S., and Landis, Q.  
1932 Vegetable amylases. Study of diastase action in the absence of maltose. *J. Am. Chem. Soc.* **54**: 216-217.
- St. John, J. L., and Bailey, C. H.  
1929 Effect of dry skim milk on the fermentation and hydrogen-ion concentration of doughs. *Cereal Chem.* **6**: 51-59.
- Working, E. B.  
1929 Fermentation tolerance. *Cereal Chem.* **6**: 506-512.

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## A METHOD FOR THE DETERMINATION OF NONPROTEIN NITROGEN IN SOYBEAN MEAL

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(Received for publication March 9, 1940)

There is no sharp, clear-cut line of demarcation between proteins and the nitrogenous nonproteins, and the more or less arbitrary analytical differentiation between them depends on the method used. Phosphotungstic and trichloroacetic acids are probably used as widely as any of the protein precipitants, and they also represent the two extremes with respect to precipitating power. Trichloroacetic acid has been used in concentrations ranging from 1% to 10%, apparently without much attention to the effect of the different acid concentrations. Cristol and Monnier (1936) state that, with blood plasma, a solution containing a final trichloroacetic acid concentration of 2.4% (Lefaux's method) leaves an appreciable amount of protein unprecipitated, an acid concentration of 2.7% (Goiffon and Spaey's method) leaves less protein unprecipitated, while a solution with 10% acid concentration (Moog's method) is protein-free. Hiller and Van Slyke (1922) state that trichloroacetic acid in concentrations of 5% or less does

<sup>1</sup> A cooperative organization participated in by the Bureaus of Agricultural Chemistry and Engineering and Plant Industry of the U. S. Department of Agriculture, and the Agricultural Experiment Stations of the North Central States of Illinois, Indiana, Iowa, Kansas, Michigan, Minnesota, Missouri, Nebraska, North Dakota, Ohio, South Dakota, and Wisconsin.

not precipitate peptones or any of the smaller protein fragments, whereas phosphotungstic acid precipitates peptones, polypeptides, and some of the amino acids. Thomas (1927), working with apple leaves and twigs, found that the precipitation of proteins from a hot aqueous extract with acetic acid, trichloroacetic acid, and colloidal ferric hydroxide gave comparable results. Copper hydroxide gave results which were quite different, because it precipitated polypeptides and some amino acids along with the protein.

Trichloroacetic acid was suggested as a protein precipitant by Greenwald (1915) 25 years ago. It is exceedingly soluble in water, and any excess is easily removed by decomposition into chloroform and carbon dioxide in hot solution. Polypeptides are not precipitated by it and proteins are not hydrolyzed even by a large excess of moderately concentrated acid.

### Proposed Method

The method herein proposed was evolved from the experimental work which is presented later in the paper. After investigating the effect of the acid concentration, the particle size and oil content of the meal, and the variety of the bean upon the values obtained for nonprotein nitrogen, the following method is proposed for its determination:

A 1-g. sample of meal is weighed into a 200-ml. centrifuge bottle and extracted with 40 ml. of 0.8*N* (13.6%) trichloroacetic acid for  $\frac{1}{2}$  hour in a mechanical shaker. The suspension is centrifuged for 5 to 6 minutes at a maximum R.C.F. of 1,975 times gravity and a 25-ml. aliquot of the clear supernatant liquid is used for a nitrogen determination by the Kjeldahl-Gunning-Arnold method. Fat-free meal is preferable but not necessary if the oil content is known for purposes of calculation or if the results are to be expressed in terms of the whole bean. The meal should be no coarser than that produced by grinding through the 1-mm. screen of a Wiley mill. The screen analysis of a fat-free meal ground in this manner is as follows: 13.4% is retained on the 35-mesh screen, 47.2% on the 60-mesh, 14.0% on the 80-mesh, 4.7% on the 100-mesh, and 20.5% passes through the 100-mesh screen.

### Effect of Acid Concentration

The beans used in the first part of this work were of the Dunfield variety grown at Lafayette, Indiana, during 1937. They were flaked and extracted with petroleum ether (boiling range 35°–60°C.) in a modified Soxhlet extractor until free from fat. The flakes were then ground in a ball mill to pass through a 100-mesh sieve. The moisture content of this meal was 9.50%, and the nitrogen content, 7.75%.

Smith and Circle (1938) extracted fat-free soybean meal with trichloroacetic acid and found that the minimum nitrogen extraction at the

isoelectric point (pH 4.1) was followed by a rise and then a second minimum as the acid concentration was increased. More concentrated acid ranges were investigated by the extraction technique described in a publication by Nagel, Becker, and Milner (1938). A  $2\frac{1}{2}$ -g. sample of fat-free meal was extracted for  $\frac{1}{2}$  hour with 100 ml. of solvent and the entire sample used for a nitrogen determination. The results of these extractions are given in Table I and represented

TABLE I  
NITROGEN EXTRACTED <sup>1</sup> BY TRICHLOROACETIC ACID OF DIFFERENT CONCENTRATIONS

Acid normality	Milligrams of nitrogen extracted per gram of meal
0.2	5.01
0.3	3.94
0.5	3.36
0.65	3.14
0.75	3.27
0.85	3.16
1.0	3.19
1.2	3.31
1.7	4.11
4.0	73.9

<sup>1</sup> Total nitrogen present was 77.8 mg. per gram of meal.

graphically in Figure 1. The first portion of the curve in Figure 1 is from the data of Smith and Circle and is reproduced here to show the

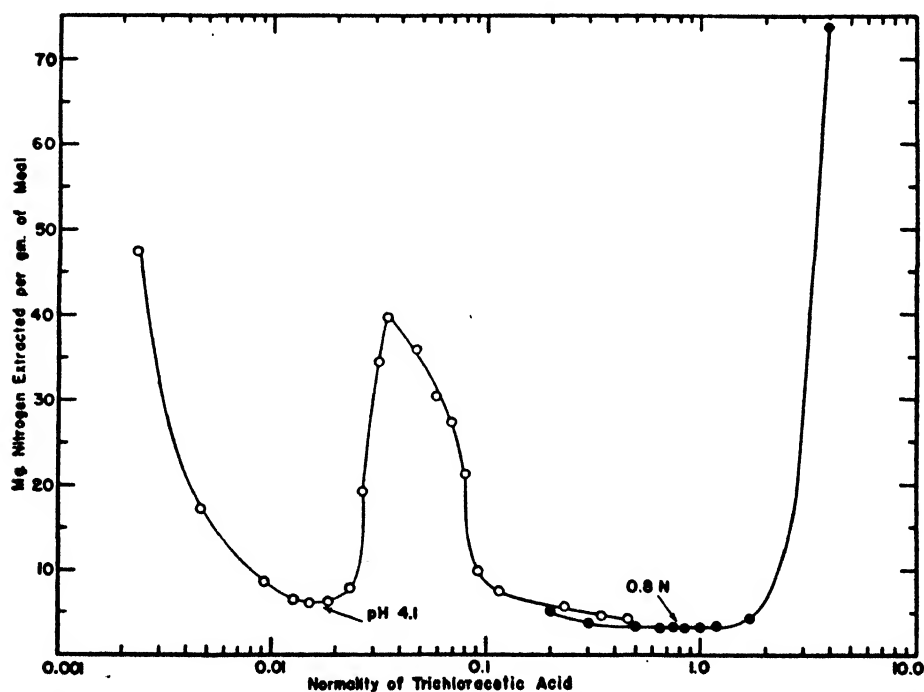


Fig. 1. Nitrogen extracted by trichloroacetic acid of various concentrations. (The curve represented by open circles is from the data of Smith and Circle. The scale of the abscissa is in logarithmic units. Total nitrogen present was 77.8 mg.)

course of the extractions over the entire acid range. The two curves do not agree exactly because two different bean samples were used. The beans used by Smith and Circle were of the Illini variety and contained 7.80% nitrogen and 9.28% moisture. The 0.8*N* acid extraction lies approximately in the center of the extraction minimum extending from 0.65*N* to 1.0*N* acid, so 0.8*N* trichloroacetic acid was chosen as the extracting agent.

The possibility of the adsorption of large amounts of nonprotein nitrogen from the 0.8*N* acid extract by the protein residue was investigated. The aqueous extract from 2½ g. of meal was centrifuged, and the proteins were precipitated from the solution at pH of 4.1. The protein was carefully washed and purified by reprecipitating several times. The solution of this protein in 0.004*N* NaOH was assumed to be relatively free from nonprotein nitrogen. Water containing glycine (7.2 mg. N), glutamic acid (8.8 mg. N), and arginine monohydrochloride (10.7 mg. N) was added to the protein solution, and the mixture was shaken thoroughly. The glutamic acid lowered the pH to 4.25 and caused coagulation of the protein, which was filtered off, and a Kjeldahl nitrogen determination was made on the filtrate. It was found to contain 26.2 mg. of nitrogen. Another protein sample was treated in the same manner except that acetic acid was used to bring the pH to 4.25. This filtrate contained only 0.4 mg. of nitrogen. Thus, all but 3.4% of the added amino nitrogen was recovered in the filtrate.

### Electrodialysis of Meal Extracts

For the purpose of nonprotein nitrogen determinations, differences in molecular size are of more significance than chemical differences. Some physical method of separation according to molecular size would be the best means of classifying the extracted nitrogenous compounds. Electrodialysis was the method chosen for this purpose, and in this work nonprotein nitrogen and the nitrogen which will electrodialyze through a cellophane membrane are taken as synonymous. Nonprotein nitrogenous compounds are here defined, therefore, chiefly on the basis of particle size.

The cell used was of glass and had ground glass faces between the three sections. The capacity of the center section was 85 ml. and that of the two end sections was about 40 ml. each. The electrodes were platinum disks 38 mm. in diameter placed about 2 mm. from the membranes. Since the anode and cathode wash liquids were to be analyzed for small amounts of nitrogen, it was necessary to keep the volumes of these liquids as small as possible. This was done by means of a still and a recirculating device which permitted the use of as little as 250

to 300 ml. of water in each chamber during a dialysis which lasted as long as five days. A cross-section drawing through the anode compartment is given in Figure 2 and a detailed section of the dialysis cell in Figure 3.

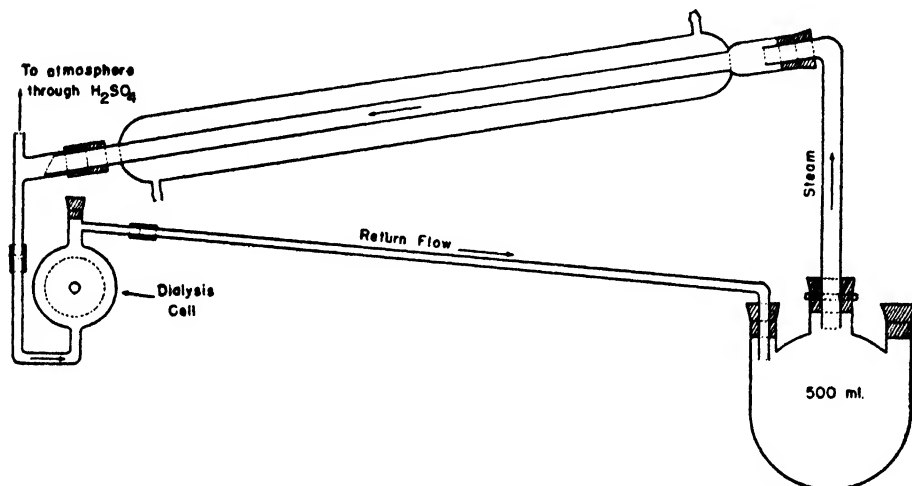


Fig. 2. Section diagram through anode compartment showing recirculating device.

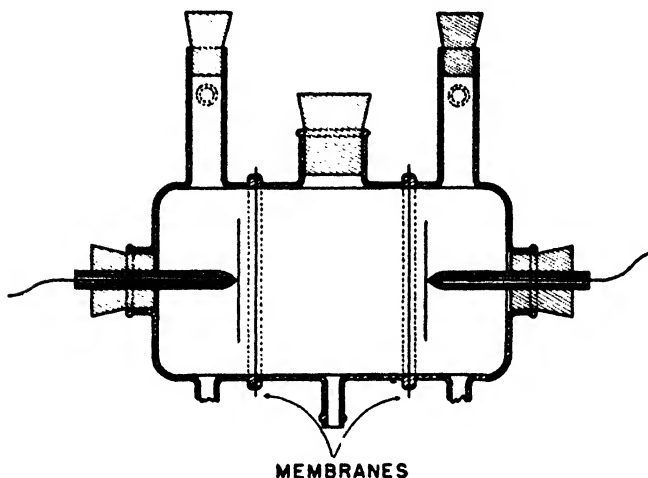


Fig. 3. Section through dialysis cell showing membranes and electrodes.

The membranes used in this work were of cellophane made especially for dialytic purposes by E. I. du Pont de Nemours and Co. A number of experiments were carried out to ascertain the approximate limit to the size of the molecules which would dialyze through this membrane. These experiments indicated that the compounds which dialyzed through were not limited to amino acids and simple peptides, but that higher-molecular-weight nonprotein compounds such as peptones could also penetrate the cellophane.

In the dialysis of the trichloroacetic acid extracts, the solutions used were the clear, supernatant liquids obtained by centrifuging the meal suspensions. Enough resistance was included in the circuit so that the current flowing through the apparatus was never in excess of 0.2 ampere. The dialysis was considered to be finished when only 5 to 7 milliamperes flowed under a potential of 220 volts, since with doubly distilled water in the cell the current was 3.5 milliamperes. The time required for dialysis was, in general, directly proportional to the amount of trichloroacetic acid added and ranged from 18 hours to 5 days.

At the conclusion of the dialysis, the solution in the center cell was removed, any precipitated protein filtered off, and the filtrate made to a volume of 100 ml. Aliquots of this solution and of the anode and cathode wash liquors, which had been concentrated to 100 ml., were used for duplicate micro-Kjeldahl nitrogen determinations. The rest of the solution from the center cell was treated with 20% phosphotungstic acid, and a nitrogen determination was made on the supernatant liquor obtained by centrifuging. The nitrogen precipitated by the phosphotungstic acid was assumed to be present principally in dispersed proteins and large protein fragments, while the nitrogen remaining in solution was nonprotein nitrogen that would have passed through the membrane if the dialysis could have been continued long enough. Basic nitrogenous nonprotein compounds were, of course, precipitated along with the protein.

TABLE II

DISTRIBUTION OF NITROGEN<sup>1</sup> AFTER ELECTRODIALYSIS OF VARIOUS TRICHLOROACETIC ACID EXTRACTS OF A SAMPLE OF DUNFIELD SOYBEANS

	EXTRACTS								Suspension of meal in water (pH 6.50)
	pH 0.3 (0.8N)	pH 4.10 (0.015N)	pH 1.25 (0.080N)	pH 1.60 (0.058N)	pH 4.92 (0.009N)	pH 5.55 (0.005N)	pH 5.85 (0.002N)	pH 5.90 (0.002N)	
Anode solution	0.75	0.70	0.64	0.97	1.00	1.11	1.60	1.54	0.80
Cathode solution	2.27	2.16	2.48	2.26	2.39	2.34	2.46	2.72	2.01
Center solution	0.17	1.90	1.27	1.06	2.46	4.04	3.42	3.52	0.72
Precipitate in center	—	1.22	5.00	30.52	3.91	29.78	29.15	35.38	—
Total recovered	3.19	5.98	9.39	34.81	9.76	37.27	36.63	43.16	—
Total extracted	3.19	6.01	9.46	34.50	9.52	36.80	37.30	44.00	—

DISTRIBUTION OF DIALYZABLE OR NONPROTEIN NITROGEN									
Anode	0.75	0.70	0.64	0.97	1.00	1.11	1.60	1.54	0.80
Cathode	2.27	2.16	2.48	2.26	2.39	2.34	2.46	2.72	2.01
Center soln. after phosphotungstic acid precipitation	0.07	0.32	0.22	0.26	0.36	0.88	0.58	0.55	0.39
Total	3.09	3.18	3.34	3.49	3.75	4.33	4.64	4.81	3.20

<sup>1</sup> Milligrams of nitrogen per gram of meal. Total nitrogen present was 77.8 mg.

Meal extracts obtained at eight different acid concentrations were dialyzed in this manner and the distribution of nitrogen in the three chambers determined. These results are presented in Table II. The last column of figures was obtained by dialyzing a suspension of the meal in water without any preliminary separation by centrifuging.

### Effect of Particle Size and Oil Content

Before an extraction procedure of this type could be of any value as an analytical method, the effect of the oil content and particle size of the meal had to be investigated. Some of the original fat-free, flaked meal was ground to different sizes in the Wiley mill, and the results obtained by extracting with 0.8*N* trichloroacetic acid are shown in Table III.

TABLE III  
EFFECT OF MEAL SIZE ON 0.8*N* TRICHLOROACETIC ACID EXTRACTION

Meal grind	Milligrams of N extracted per gram of meal
Flakes	3.03
2-mm. screen of Wiley mill	3.00
1-mm. screen of Wiley mill	3.09
$\frac{1}{2}$ -mm. screen of Wiley mill	3.14
100 mesh meal (ball mill)	3.19

Total nitrogen present was 77.8 mg.

The amount of nitrogen extracted from the 1-mm. Wiley screen meal is about 3% less than that extracted from the 100-mesh meal, but since all of the routine samples of the U. S. Regional Soybean Industrial Products Laboratory are ground to the former size, it was used in all subsequent work.

To determine the effect of the oil, three samples of 1-mm. Wiley screen meal of different oil content were extracted with 0.8*N* trichloroacetic acid. The results, given in Table IV, show that the oil has no effect at all except to change the basis for calculating results.

TABLE IV  
EFFECT OF OIL CONTENT ON TRICHLOROACETIC ACID EXTRACTION

	Oil	0.8 <i>N</i> extract	Oil-free basis
	%	mg. <i>N</i> extd./g. meal	mg. <i>N</i> extd./g. meal
Whole meal	21.5	2.50	3.19
Percolator extracted	3.0	3.05	3.15
Soxhlet extracted	0.0	3.20	3.20

Total nitrogen present was 77.8 mg.



Since trichloroacetic acid is a rather expensive reagent, it was thought advisable to determine whether smaller quantities could be used. The results were found to be the same and equally reproducible when 1 g. of meal was extracted with 40 ml. of 0.8*N* trichloroacetic acid and a 25-ml. aliquot of this used for the nitrogen determination. The use of the 25-ml. portion is also advantageous in that there is much less trouble with foaming during the Kjeldahl digestion.

### Trichloroacetic Acid Extraction of Different Soybean Meals

A number of samples of different beans were extracted by the above-mentioned procedure to determine whether any difference in non-protein nitrogen could be observed between the different varieties and localities of growing. A dozen samples were selected to represent, so far as possible, a high- and a low-nitrogen bean of each of several varieties. A sample of Dunfield beans was sprouted in the dark, dried at 105°C., ground in the Wiley mill, defatted by a percolator extraction, and then extracted with 0.8*N* trichloroacetic acid. A control sample was treated in the same manner, except that it was not sprouted. These results and a short description of each bean are given in Table V, which also includes the nitrogen, oil, and moisture figures on the whole bean.

TABLE V  
EXTRACTION OF DIFFERENT BEAN SAMPLES WITH 0.8*N* TRICHLOROACETIC ACID

Samples	Fat-free meal			Whole meal		
	Total nitrogen in a gram of meal	0.8 <i>N</i> extract		Nitrogen	Oil	Moisture
		mg. N/g. meal	Percent of total nitrogen extracted			
	mg.			%	%	%
No. 267—Mukden 1936—Ames, Iowa	90.3	7.04	7.80	7.68	16.48	6.45
No. 499—Mukden 1937—Lafayette, Ind.	84.9	3.10	3.65	7.07	17.30	5.75
No. 507—Dunfield 1937—Columbus, Ohio	73.2	2.11	2.88	6.00	19.50	5.48
No. 510—Mukden 1937—Columbus, Ohio	79.8	2.30	2.88	6.77	18.08	5.78
No. 517—Mukden 1937—Ames, Iowa	83.1	3.92	4.72	7.00	18.39	5.83
No. 520—Dunfield 1937—Ames, Iowa	76.0	2.78	3.66	6.13	19.92	5.74
No. 632—Illini 1937—Osceola, Ark.	66.2	3.07	4.64	5.03	23.79	5.53
No. 655—86518—1937—Ames, Iowa	85.7	6.38	7.44	7.50	15.15	6.10
No. 789—Dunfield 1937—Ames, Iowa	70.3	2.61	3.71	5.72	21.24	5.85
No. 932—Mukden 1937—Ames, Iowa	78.0	2.74	3.51	6.54	18.60	5.77
No. 935—Dunfield 1937—Sand Expt. Field, Ind.	79.3	5.02	6.33	6.89	14.47	6.00
No. 937—Dunfield 1937—Lafayette, Ind.	73.0	2.50	3.42	6.07	20.64	5.67
Ungerminated—Dunfield 1937	77.8	2.81	3.61	—	—	—
Germinated—Dunfield 1937	77.8	5.63	7.24	—	—	—

The lack of correlation between the amount of total nitrogen present in the bean and the amount removed by 0.8*N* trichloroacetic acid indicates that a definite type of nitrogenous material is extracted. As a further check, the two samples highest in nonprotein nitrogen, the two

lowest, and one medium sample were extracted at a pH of about 4.1 and the extracts were electrodialyzed. The results, presented in Table VI, indicate a very definite relationship between the amounts of nitrogen extracted by 0.8*N* trichloroacetic acid and the amounts of nitrogen which will electrodialyze through the cellophane membranes.

TABLE VI  
THE RESULTS<sup>1</sup> OF DIALYSIS OF PH 4.1 TRICHLOROACETIC ACID  
EXTRACTS OF VARIOUS BEAN SAMPLES

	Sample numbers						
	267	655	632	507	510	Germinated	Ungerminated
Anode compartment	1.03	1.43	1.03	0.78	0.73	1.15	0.66
Cathode compartment	5.86	4.76	2.39	1.66	1.63	3.89	2.20
Center compartment (not pptd. by phosphotungstic acid)	0.26	0.24	0.18	0.16	0.16	0.26	0.34
Total nonprotein nitrogen by electrodialysis	7.15	6.43	3.60	2.60	2.52	5.30	3.20
Total nitrogen extracted by 0.8 <i>N</i> trichloroacetic acid	7.04	6.38	3.07	2.11	2.30	5.63	2.81
Total nitrogen in meal	90.3	85.7	66.2	73.2	79.8	77.8	77.8

<sup>1</sup> Expressed in milligrams of nitrogen per gram of meal.

### Discussion

The 0.8*N* trichloroacetic acid extraction lies in approximately the center of the broad minimum of the curve of nitrogen extracted (Fig. 1) extending from 0.65*N* to 1.0*N* acid concentrations. The curve in this zone is practically a straight line and even rather large differences in acid concentration would cause no appreciable error in the amount of nitrogen extracted in this concentration range. The amount of nitrogen extracted in this minimum range is only half that removed at pH 4.1, and no protein precipitate was ever observed in the center cell after dialysis of one of these 0.8*N* extracts, whereas some precipitated protein was always observed when an extract made at pH 4.1 was used. It is assumed that the other extracts made with trichloroacetic acid at concentrations between 0.5*N* and 1.0*N* would behave similarly upon dialysis. The almost complete recovery of the amino nitrogen added to the purified protein solution indicates that very little adsorption occurs in the extraction procedure. It is true, of course, that the conditions are not exactly parallel, but it would be very difficult to devise any experiment in which the protein is retained in its native form.

The dialysis of the 0.8*N* trichloroacetic acid extract (Table II) shows that practically all of the nitrogen extracted is of such a nature that it will pass through the membrane. The trace of nitrogen which is left in the center would very probably dialyze through also if a sufficiently long time were allowed. No satisfactory explanation can be offered

at the present time for the increasing amounts of dialyzable nitrogen in the higher pH extracts. It is felt, however, that since the 0.8*N* acid extract, the pH 4.1 extract, and the meal-water suspension give results which check very closely, the other anomalous results do not invalidate the conclusion that the 0.8*N* acid extract contains no nitrogen but nonprotein nitrogen and practically all of the nonprotein nitrogen.

Since the 12 samples of beans selected for extraction (Table V) were grown at agricultural experiment stations under different conditions, it seems very reasonable to assume that the different varieties of beans should contain widely varying amounts of nonprotein nitrogen. There is no correlation between the amount of total nitrogen in the bean and that extracted. This fact also serves to indicate that some definite type of nitrogen or some nitrogen fraction is being preferentially extracted, and that it is not just a random solution of some of the protein. The dialysis of extracts of five of these samples made at a pH of about 4.1 (Table VI) shows that all of the nitrogen extracted by 0.8*N* trichloroacetic acid and only that nitrogen will electro dialyze through the cellophane membranes.

### Summary

In the extraction of soybean meal with trichloroacetic acid, the minimum amount of nitrogen was removed with acid concentrations lying between 0.65*N* and 1.0*N* acid, 0.8*N* acid being taken as the midpoint. The amount of nitrogen extracted in this region was only about one-half that extracted at pH 4.1, which is the isoelectric point of soybean protein in the meal as determined by solubility measurements.

An electro dialysis apparatus, in which the water is recirculated after purification by distillation, is described. This arrangement has the advantage of keeping the volume of water circulated through the electrode chambers at a minimum.

Soybean meal was extracted with trichloroacetic acid of eight different concentrations and the extracts electro dialyzed through cellophane membranes. All of the nitrogen extracted with 0.8*N* acid dialyzed through the membrane while all other extracts left some precipitated protein in the center compartment.

Several varieties of beans grown in different localities were extracted with 0.8*N* trichloroacetic acid. The nonprotein nitrogen determinations indicated relatively large differences and bore no relationship to the total nitrogen present. Electro dialysis of extracts of five of these samples showed again that the amount of nitrogen extracted with 0.8*N* acid and the amount which electro dialyzed are of the same magnitude.

Details are given for a simple, rapid procedure which should serve as a routine method for determining nonprotein nitrogen in soybean meal. The nonprotein nitrogen is extracted with 0.8*N* trichloroacetic acid and the nitrogen in the extract is determined directly.

#### Literature Cited

- Cristol, P., and Monnier, P.  
1936 Deproteinization of serum by trichloroacetic acid for the determination of total nonprotein nitrogen. *Compt. rend. soc. biol.* **123**: 1106-1107.
- Greenwald, I.  
1915 The estimation of nonprotein nitrogen in blood. *J. Biol. Chem.* **21**: 61-68.
- Hiller, A., and Van Slyke, D. D.  
1922 A study of certain protein precipitants. *J. Biol. Chem.* **53**: 253-267.
- Nagel, R. H., Becker, H. C., and Milner, R. T.  
1938 Some physical factors affecting the dispersion of soybean proteins in water. *Cereal Chem.* **15**: 463-471.
- Smith, A. K., and Circle, S. J.  
1938 Peptization of soybean proteins: extraction of nitrogenous constituents from oil-free meal by acids and bases with and without added salts. *Ind. Eng. Chem.* **30**: 1414-1418.
- Thomas, W.  
1927 Influence of temperature of desiccation on water-soluble nitrogenous constituents and separation of water-soluble protein from nonprotein constituents. *Plant Physiol.* **2**: 55-66.

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## THE HEMICELLULOSES FROM OAT HULLS. II

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(Received for publication December 18, 1939)

In a former paper (Anderson and Krznarich, 1935) it was intimated that a mixture of hemicelluloses was obtained from oat hulls. Furthermore, results seemed to indicate the presence of a polyose type of hemicellulose along with a polyuronide type. By using refinements in methods of preparation, purification, and fractionation, the author, in a continuation of work along these lines, has obtained data which show a definite separation of the two types of hemicelluloses.

#### Experimental

*Preparation of hemicelluloses A, B, and C.*—Five hundred grams of oat hulls were extracted repeatedly with acetone, hot alcohol, and boiling water. The hulls were then mixed with 7 times their weight of 5% sodium hydroxide solution and allowed to stand for 36 hours. The mixture was filtered and hemicellulose *A* was precipitated from the solution by slightly acidifying with hydrochloric acid. The precipitate was separated and completely washed in the centrifuge. Hemicellulose *B* was precipitated from the filtrate from *A* by addition of 1½ volumes of ethyl alcohol (80%-90%). The residual hulls were extracted a

second time with sodium hydroxide, and a second crop of both hemicellulose *A* and *B* was obtained in a similar manner. These will be referred to as *A'* and *B'*. The residual hulls were then extracted with 0.05 *N* hydrochloric acid, and pectin *A* was thus removed. An extraction with 5% ammonium hydroxide removed pectin *B* (Anderson, 1936). The hulls were then placed in a weak acid solution and chlorinated for one hour. Upon extraction with hot alcohol a supposed chlorine compound of lignin was removed. This was precipitated from the alcohol solution by addition of water. This material resembled that extracted from wood sawdust (Anderson, unpublished data). The residual hulls were extracted again with 5% sodium hydroxide solution and hemicellulose *C* was removed and precipitated as before. No pectic material was obtained after chlorination of the hulls. The total yield of hemicellulose was approximately 35% of the weight of the hulls used. This is a 7% increase over the amount previously reported (Anderson and Krznarich, 1935).

*Fractionation and purification of the hemicelluloses.*—Crude hemicellulose *A*, which showed a carbon dioxide content of 0.77% (Lefevre and Tollens, 1907; Dickson, Otterson, and Link, 1930), a pentosan of 92.49%, ash of 1.31%, and  $[\alpha]_D^{20}$  in 5% sodium hydroxide of  $-78.21^\circ$ , was purified by chlorination. This resulted in a hemicellulose *A* with a carbon dioxide content of 0.73%, a pentosan of 95.56%, and ash of 0.60%. The hemicellulose was dissolved in 5% sodium hydroxide solution, the solution made just acid, and chlorine gas passed in with shaking for 30 minutes. The mixture was centrifuged and the most insoluble fraction, hemicellulose *A*<sub>1</sub>, was removed. The second fraction was obtained by adding 1½ volumes of alcohol to the filtrate from *A*<sub>1</sub>. Each hemicellulose, both from the first and second sodium hydroxide extractions, was treated in the same way. This resulted in fractions *A*<sub>1</sub>, *A*<sub>2</sub>, *A*<sub>1</sub>', *A*<sub>2</sub>', *B*<sub>1</sub>', *B*<sub>2</sub>'. Hemicellulose *B* was not fractionated since only a small amount of it was originally obtained. Results of analyses are given in Table I.

In each case a distinct fractionation is evident. Hemicellulose *A* is split into a fraction *A*<sub>1</sub>, which is evidently almost a pure xylan. Although the carbon dioxide content is 0.56% (corresponding to uronic anhydride of 2.24%), it was found difficult to prepare any polyose material which gave a carbon dioxide value radically less by the method used. Mannans *A* and *B*, prepared from ivory nut waste, gave carbon dioxides of 0.45% and 0.53% respectively. Fractions *A*<sub>1</sub>' and *A*<sub>2</sub>' give analytical values corresponding to that of the above fraction and may be termed polyose materials. Fractions *A*<sub>2</sub>, *B*, *B*<sub>1</sub>', and *B*<sub>2</sub>' are undoubtedly polyuronide materials, as evidenced by the increased uronic anhydride content.

TABLE I  
RESULTS OF PRELIMINARY PURIFICATION AND FRACTIONATION

Fraction	Carbon dioxide	Pentosan	$[\alpha]_D^{20}$ in sodium hydroxide	Ash
A.....	0.73%	95.56%	-78.21°	0.60%
A <sub>1</sub> .....	0.56%	97.35%	*	1.67%
A <sub>2</sub> .....	1.85%	80.93%	-83.03°	*
B.....	1.49%	66.93%	*	*
A <sub>1</sub> '.....	0.54%	*	*	7.38%
A <sub>2</sub> '.....	0.63%	*	*	*
B <sub>1</sub> '.....	0.84%	*	*	*
B <sub>2</sub> '.....	1.22%	*	*	*
C.....	0.72%	*	*	*

\* Values not obtained.

Further treatment of hemicellulose *A*<sub>1</sub> by redissolving in sodium hydroxide solution, chlorinating, and reprecipitating gave two fractions containing 0.67% and 1.75% carbon dioxide respectively. The fraction analyzing 0.67% carbon dioxide was of the higher yield, approximately 55% of the original, the second being 10%.

By extracting fractions *A*<sub>2</sub>', *C*, and *B*<sub>1</sub>' with hot water, it was possible to separate each into a water-soluble and water-insoluble portion. Hemicellulose *B*<sub>2</sub>' was entirely water soluble. The results of carbon dioxide determinations on each of the fractions: hemicellulose *A*<sub>2</sub>', water soluble 0.94%, water insoluble 0.80%; hemicellulose *C*, water soluble 0.90%, water insoluble 0.61%; hemicellulose *B*<sub>1</sub>', water soluble 1.21%, water insoluble 0.85%. In each case the water-soluble fraction shows a higher uronic anhydride content substantiating the results obtained above (Table I). From these data it is readily ascertainable that polyuronides are more soluble and more easily extracted than the polyoses.

### Summary

Repeated fractionation of the hemicelluloses from oat hulls showed them to be composed of polyose and polyuronide material, the polyuronide type being more soluble and more readily extracted.

### Literature Cited

- Anderson, E., and Krznarich, P. W.  
1935 Hemicellulose from oat hulls. *J. Biol. Chem.* **111**: 549-552.
- Anderson, E.  
1936 The isolation of pectic substances from wood. *J. Biol. Chem.* **112**: 531-539.
- Dickson, A. D., Otterson, H., and Link, K. P.  
1930 A method for the determination of uronic acids. *J. Am. Chem. Soc.* **52**: 775-779.
- Lefevre, K. U., and Tollens, B.  
1907 Untersuchungen über die Glukuronsäure. *Ber. Chem. Ges.* **40**: 4513.

# DOUGH IMPROVEMENT STUDIES. I. OXIDATION OF GLUTATHIONE BY POTASSIUM BROMATE

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(Received for publication November 13, 1939)

The presence of glutathione was actually discovered about 50 years ago. It was rediscovered and identified in 1921 by F. G. Hopkins. Glutathione is composed of three amino acids, cystine, glutamic acid, and glycine, which occur in wheat gliadin to the extent of 2.2%, 43.0%, and 0.0% and in wheat glutenin to the extent of 3.2%, 26.5%, and 0.9% (Ritter, 1939). This compound exists in a reduced form (henceforth referred to as GSH) with an open thiol-group (-SH) and in an oxidized form (to be referred to as GSSG) with an S-S bridge. An excellent survey of our knowledge of glutathione is to be found in a French monograph by Binet and Weller (1937).

It has been pointed out that "low-gradeness of flour" does not depend on the ether-extractable fraction of wheat, but on some other factor or factors. Among non-enzymatic substances proved to have a more or less deleterious effect on baking quality are asparagine (Bull, 1937) and, according to Sullivan *et al.* (1936, 1936a, and 1937), trimethylamine, guanidine and especially glutathione. Jørgensen (1935, 1935a) has already drawn attention to the importance of glutathione in baking.

Since the work of Grassmann in 1931 it is known that GSH activates the proteolytic enzymes of the papain type, which is the type found in wheat, according to the investigations of Balls and Hale (1935, 1936, 1938). Ford and Maiden (1938) interpret their results as showing that it is not activation of the papain that happens through the presence of GSH in a dough, but that most likely there is a direct action on the gluten proteins. Balls and Hale (1936a) also mention that possibly GSH activates the gluten proteins. In this connection it is interesting to note the suggestion of Albers (1936) that the activation of the papain by GSH takes place on the apo-enzyme, which would be the protein component of the enzyme. Glutathione may influence fermentation rate, but all authors do not yet agree on this issue.

An important role of glutathione is the maintenance of the reducing activity of the cell, which seems to be necessary since synthesis in the cell is a reduction process (Bertho, 1935), whereas oxidation leads to the breaking down of cell compounds. Van Laer (1935) mentions that after death the rH increases. GSH has in fact an exceptionally strong reducing power. At pH 7.0 and 37° for a 0.01 molar solution an rH as low as 9.3 has been found, whereas measurements on wheat-flour

doughs gave an rH of 15 to 18 (Van Laer, 1935a; Potel and Chaminade, 1935). For a convenient explanation of the term rH the reader is referred to Kent-Jones (1939).

Other reducing substances in a wheat-flour dough are some of the sugars present of which levulose (a component of sucrose) has specially strong reduction properties. Schoen (1938) states that the rH of glucose (dextrose) and levulose are 11.3 and less than 2.3 respectively. The latter may—at some stage in the process of fermentation—exert its influence before being itself fermented.

### Experimental

The glutathione (GSH) was obtained from Hoffmann, La Roche, in Basle (Switzerland). The potassium bromate ( $\text{KBrO}_3$ ), the maleic acid, and the copper and iron salts were normal Merck products. The buffer solutions were prepared according to Sørensen by mixing varying amounts of a 1/15 molar solution of primary potassium phosphate ( $\text{KH}_2\text{PO}_4$ ) with varying amounts of a 1/15 molar solution of secondary sodium phosphate ( $\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$ ).

A micro iodine titration, using starch as indicator, was used to follow the oxidation of glutathione. The reaction is  $2\text{GSH} + 2\text{I} = \text{GSSG} + 2\text{HI}$ . The molecular weight of GSH is 307, so that:

1.000 cc. n/500 iodine corresponds to 0.614 mg. GSH  
or: 1.628 cc. n/500 iodine corresponds to 1.000 mg. GSH

Treadwell (1927) draws attention to the fact that concentration is of importance in titrations with weak iodine solutions; he reports that the blue coloration appeared after adding 0.15 cc. iodine solution when 50 cc. water was used, but only after adding 0.64 cc. when 200 cc. of water was used.

### Rate of Oxidation with Bromate

The oxidation was followed for several hours using different concentrations of bromate (Fig. 1). GSH in water solution was slowly autoxidized. After 21 hours only roughly 60% of the glutathione was still present in the reduced form.

Not even with 50 times more bromate than GSH (curve 6) was the oxidation instantaneous. The fact that curve 7 (Fig. 1) never quite touched the abscissa is no doubt due to the situation observed by Treadwell (1927) as already mentioned. It is interesting to note that all along the oxidation with the highest concentration of bromate (curve 7) required only 0.05 cc. of n/500 iodine, whereas in all other cases (Figs. 1 and 2; Table II) a minimum of 0.15 cc. was required. Pure water alone needed 0.22 cc. iodine solution.



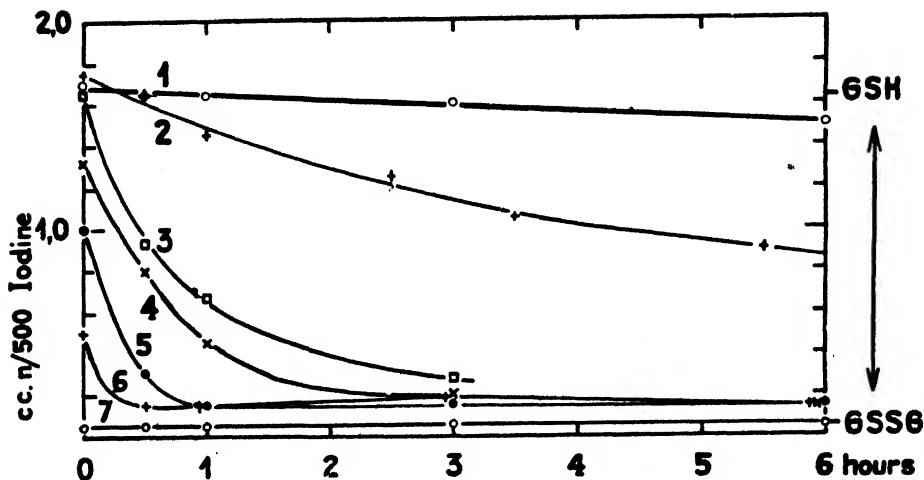


Fig. 1. Rate of oxidation of glutathione (GSH) by different amounts of bromate at room temperature. In all cases 1 mg. GSH in 10 cc. distilled water was used. The ratios of GSH to  $\text{KBrO}_3$  were:

Curve 1, 1:0	Curve 3, 1:5	Curve 5, 1:20	Curve 7, 1:500
Curve 2, 1:0.5	Curve 4, 1:10	Curve 6, 1:50	

After 21 hours the cc. of n/500 iodine required for the conditions represented by the respective curves were as follows: curve 1, 1.00; curves 4, 5, and 6, 0.10; curve 7, 0.05, and curve 2 (after 8 hours), 0.7 cc.

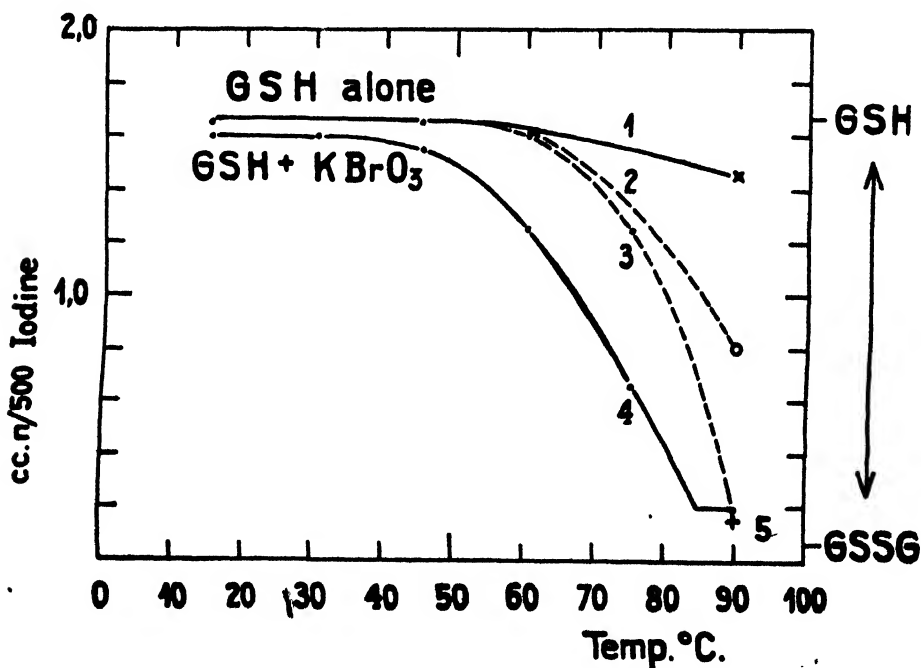


Fig. 2. Influence of temperature on the oxidation of 1 mg. glutathione (GSH) by 0.5 mg. bromate in 10 cc. distilled water. Reaction time 10 minutes. Cooled before titration.

Curve 1, GSH alone in  $\text{CO}_2$  saturated distilled water.  
 Curve 2, GSH alone in boiled distilled water.  
 Curve 3, GSH alone in distilled water, not previously boiled.  
 Curve 4, GSH +  $\text{KBrO}_3$  in distilled water, not previously boiled.  
 Point 5, GSH +  $\text{KBrO}_3$  in  $\text{CO}_2$  saturated distilled water.

### Influence of Hydrogen-Ion Concentration

According to Harington and Mead (1935) an aqueous solution of GSH has a pH of about 2.5. Read and Haas (1938) state that free bromic acid is formed from bromate only at a very low pH.

TABLE I

INFLUENCE OF pH ON THE OXIDATION OF GLUTATHIONE (GSH) SOLUTION ALONE  
(Buffer solutions consisted of Sørensen's phosphate mixtures.  
Reaction time 5 minutes.)

pH	10 cc. buffer solution + 0.5 cc. KBrO <sub>3</sub>	10 cc. buffer solution + 1 mg. GSH	10 cc. buffer solution + 1 mg. GSH + 0.5 mg. KBrO <sub>3</sub>
	<i>cc. n/500 iodine</i>		
8.0	0.15	(3.30) <sup>1</sup>	(3.50)
7.0	0.10	(2.55)	(2.68)
6.2	0.10	(2.05)	(2.07)
5.6	0.10	1.75	1.75
5.0	0.10	1.75	1.60
4.5	0.10	1.75	1.65

<sup>1</sup> Parentheses denote that the exact end point of the titration was difficult to fix, because the blue coloration disappeared slowly again.

TABLE II

OXIDATION OF GLUTATHIONE (GSH) BY BROMATE IN THE PRESENCE OF  
CERTAIN SUBSTANCES AT ROOM TEMPERATURE  
(In all cases 1 mg. GSH in 10 cc. distilled water)

No.	KBrO <sub>3</sub> mg.	Addition	Reaction time in minutes		
			0	5	60
			<i>cc. n/500 iodine</i>		
1	5	None	1.70	1.60	0.70
2	5	9 mg. fresh baker's yeast	1.65	1.65	1.10
3	5	6 mg. NaCl	1.65	1.60	0.95
4	5	15 mg. NaCl	1.65	1.60	0.95
5	5	2.5 mg. copper chloride <sup>1</sup>	1.85	1.65	0.60
6	5	8.4 mg. ferric sulphate	1.25	0.20	0.20
7	5	4.0 mg. ferrous sulphate	1.20	0.15	0.15
8	5	1.0 mg. ferrous sulphate	1.35	0.25	0.25
9	0.5	None	1.65	1.65	1.45
10	0.5	2.5 mg. copper chloride <sup>1</sup>	1.80	1.60	1.25
11	0.5	8.4 mg. ferric sulphate	1.25	0.95	0.15
12	0.5	4.0 mg. ferrous sulphate	1.55	1.15	0.15
13	None	2.5 mg. copper chloride <sup>1</sup>	1.85	1.60	1.50
14	None	8.4 mg. ferric sulphate	1.40	1.00	0.60
15	None	4.0 mg. ferrous sulphate	1.60	1.60	1.60
16	None	None	1.70	1.65	1.65

<sup>1</sup> In presence of copper chloride (Nos. 5, 10, and 13) the blue coloration keeps on disappearing, making it hard to fix the end point of the titration.

Unfortunately no apparatus was at hand for the precise control of pH. The figures in Table I show that oxidation is very slow between

pH 4.5 and 5.6. Above about pH 6 probably a rapid but partial decomposition of the GSH took place. The optimum for the oxidation to GSSG by oxygen is given as pH 7.4 by Dixon and Tunnicliffe (1923). At higher pH there is said to be a cleavage of the molecule with the formation of sulphenic, sulphinic, and sulphonic acids.

### **Influence of Temperature**

It has already been mentioned that GSH alone in water autoxidized to the extent of 60% in 21 hours at room temperature (Fig. 1). The autoxidation is rapid above 60° (Fig. 2). Ten minutes at 90° is sufficient to completely oxidize GSH if water containing a certain amount of absorbed oxygen is used. If the latter is driven out by boiling previous to the addition of GSH a partial oxidation takes place, probably due on the one hand to the incomplete expulsion of all traces of oxygen, and on the other hand to the contact of the liquid surface with the atmosphere, in the presence of traces of both copper and iron compounds. Treatment at 90°C. for 10 minutes in an atmosphere of carbon dioxide considerably reduced the degree of oxidation.

In the presence of a small amount of bromate, oxidation of GSH was noticeably accelerated above 40°C. Nevertheless it needed a temperature of 85°C. to completely oxidize the GSH in that short a time. These tests again show the slowness of the oxidation of GSH by bromate as compared with oxidation by iodine, even if heat is used.

### **Influence of Some Catalysts**

Results given in Table II show that, with the amounts used and with reaction times up to one hour, neither baker's yeast nor sodium chloride had any accelerating influence on the oxidation of GSH with bromate. As was expected, yeast, which contains GSH itself, increased the amount of iodine required. It is known that copper and iron play an important part in biological oxidations. As a catalyst of the bromate oxidation copper chloride seemed to have no effect in our tests. On the other hand both  $\text{Fe}^{++}$  and  $\text{Fe}^{+++}$  salts acted as strong catalysts. The copper and ferrous ( $\text{Fe}^{++}$ ) salts alone had no action on GSH, whereas the ferric ( $\text{Fe}^{+++}$ ) salt alone oxidized roughly to the same extent as bromate did. Binet and Weller (1936) used ferric chloride to completely oxidize GSH in 24 hours.

Next it was thought that some compound in flour might activate the oxidation of a bromate-treated dough. The water-extractable part of a short-patent flour did not seem to exert any significant influence on the oxidation rate, as figures in Table III show.

TABLE III

INFLUENCE OF FLOUR EXTRACT ON THE OXIDATION OF 1 MG. GLUTATHIONE (GSH) BY 0.5 MG. BROMATE IN 10 CC. DISTILLED WATER  
(10 g. short-patent flour extracted by 50 cc. distilled water at 27°C. for 1 hour; filtration 20 minutes; in all cases 5 cc. flour extract used)

GSH	KBrO <sub>3</sub>	Reaction time	cc. n/500 iodine		
			Actual titration	Cc. used by flour extract deducted	Without flour extract (from Fig. 1)
mg.	mg.	hrs.			
—	—	0	0.60	—	—
1	—	0	2.00	1.40	1.65
1	0.5	0	1.90	1.30	1.75
1	0.5	1	1.95	1.35	1.50
1	0.5	2	1.90	1.30	1.30
1	0.5	3	1.70	1.10	1.15

### Influence of Different Oxidizing Agents and Maleic Acid

Bromate and iodate, well-known improvers in the baking industry, also completely oxidized glutathione (Table IV). Chlorate, on the other hand, is known to have no improving action in a dough and did not appear to oxidize the glutathione in the least.

TABLE IV

INFLUENCE OF A FEW OXIDIZING AGENTS AND MALEIC ACID ON THE OXIDATION OF GLUTATHIONE (GSH)  
(50 mg. of each substance dissolved in 10 cc.)

Product added	GSH	Reaction time	n/500 iodine	Degree of oxidation
	mg.	hrs.	cc.	
None	1	1	1.65	—
Potassium chlorate	1	1	1.60	No oxidation
Potassium bromate	1	1	0.15	Complete
Potassium iodate	1	1	0.15	Complete
Dehydroascorbic acid	1	1	0.80	50%
Maleic acid	—	0	0.15	No effect on iodine
Maleic acid	1	1	1.90	No oxidation

Melville and Shattock (1938) found that the oxidized form of vitamin C, dehydroascorbic acid, is just as efficient an improver as bromate. For our titration 50 mg. of ascorbic acid was first completely oxidized with iodine before being brought together with 1 mg. of glutathione. In spite of this larger amount of dehydroascorbic acid, as compared with the amount of bromate, the rate of oxidation was only half that of the bromate (Table IV).

TABLE V

## BAKING TESTS WITH BROMATE AND MALEIC ACID AS IMPROVER

(100 g. flour, 2 g. salt, 3 g. yeast, 0.5 g. malt flour, 65 cc. water; 2 hours fermentation; proof as needed)

	Wheat flour	Untreated	Bromate 2 mg.	Maleic acid		
				2 mg.	5 mg.	10 mg.
Loaf vol., cc.	Canadian	680	720	650	700	—
Loaf vol., cc.	Argentine	410	460	435	430	430
Oven spring		Fair	Excellent	Unsatisfactory		
Texture		Fine	Very fine, silky	Coarse		

Recently Morgan and Friedmann (1938, 1938a) showed that on addition of maleic acid to thiol compounds (to which class glutathione also belongs)  $\alpha$ -thiosuccinic acid derivatives are formed. Such an addition product with glutathione was isolated. Furthermore the authors found that enzymic actions induced by SH-compounds were inhibited by the addition of maleic acid ( $\text{HOOC} \cdot \text{CH} = \text{CH} \cdot \text{COOH}$ ). If that is the case, it might be possible to so bind the glutathione in dough that it can no longer activate the proteolytic enzymes of wheat flour, which, according to Jørgensen's (1935) theory, would bring about and improvement in baking quality. Table IV shows that on the addition of the acid the reducing power of glutathione was not diminished at all. Baking tests (Table V) also brought out the difference between a treatment with bromate and with maleic acid. On the addition of the latter none of the characteristics of improved doughs were observed. In a wheat-flour dough maleic acid would not seem to react according to the manner indicated by Morgan and Friedmann (1938a).

## Summary

For over 20 years potassium bromate has been widely used to improve the baking quality of wheat flour. An explanation of the reaction has only recently been given by Jørgensen (1935).

It was thought of interest to study the rate of oxidation by bromate of pure reduced glutathione, since the latter is probably the main factor responsible for the "low-gradeness of flour" (Sullivan, Howe, and Schmalz, 1936a).

Glutathione in water solution was autoxidized to the extent of 60% in 21 hours at room temperature. In a water solution saturated with carbonic acid autoxidation was slow, even at 90°C.

At temperatures below 40°C. an enormous overdose of bromate is necessary for the rapid oxidation of glutathione, as compared with iodine, which oxidizes immediately. A treatment of 10 minutes above 40°C. greatly accelerated the oxidation, which was completed in that lapse of time at 85°C.

Below a pH of 5.6 the hydrogen-ion concentration had no marked influence on the oxidation with bromate. Above pH 6.2 glutathione itself was probably broken down.

Whereas copper chloride seemed to have no effect on the rate of oxidation, ferric and ferrous sulphate both exerted a significant accelerating action on the oxidation. The water extract of flour did not influence the rate of oxidation.

Maleic acid is said to form an addition product with glutathione, inhibiting the activating properties of the latter on enzyme action. It was found that maleic acid had no influence on the reducing power of pure glutathione, nor did it communicate any of the characteristics of bromate treatment when added to a wheat-flour dough.

Bromate is known to work gradually as an improver in wheat-flour dough. This can be explained by its slow rate of oxidation of glutathione.

#### Acknowledgment

The author wishes to acknowledge the help and suggestions of Mr. F. Brassel in carrying out the work reported in this paper.

#### Literature Cited

- Albers, H.  
1936 Wesen und Wirkung der Fermente. *Angew. Chem.* **49**: 448-455.
- Balls, A. K., and Hale, W. S.  
1935 Proteolysis in flours. *J. Assoc. Offic. Agr. Chem.* **18**: 135-140.  
1936 Proteolytic enzymes of flour. *Cereal Chem.* **13**: 54-60.  
1936a Further studies on the activity of proteinase in flour. *Cereal Chem.* **13**: 656-664.  
1938 The preparation and properties of wheat proteinase. *Cereal Chem.* **15**: 622-628.
- Bertho, A.  
1935 Die biologische Zelloxydation. *Chemik.-Ztg.* **59**: 956.
- Binet, L., and Weller, G.  
1936 Glutathion total des tissus: méthode de dosage; répartition chez les animaux normaux. *Bul. soc. chim. biol.* **18**: 358-374.  
1937 Le glutathion. *Actualités scientifiques et industrielles.* Hermann et Cie., Paris.
- Bull, Joan E.  
1937 An investigation into the cause of the degrading effect of wheat germ on the baking quality of flour. *Cereal Chem.* **14**: 244-256.
- Dixon, M., and Tunnicliffe, H. E.  
1923 The oxidation of reduced glutathione and other sulfhydryl compounds. *Proc. Roy. Soc. (London)* **B94**: 266-297.
- Ford, W. P., and Maiden, A. M.  
1938 Effects in dough of glutathione and papain. *J. Soc. Chem. Ind.* **57**: 278-281.
- Harrington, C. R., and Mead, T. H.  
1935 Synthesis of glutathione. *Biochem. J.* **29**: 1602-1611.
- Jørgensen, H.  
1935 Über die Natur der Einwirkung von Kaliumbromat und analogen Stoffen auf die Backfähigkeit des Weizenmehles, I. *Biochem. Z.* **280**: 1-37.  
1935a Über die Natur der Einwirkung von Kaliumbromat und analogen Stoffen auf die Backfähigkeit des Weizenmehles II. *Biochem. Z.* **283**: 134-145.
- Kent-Jones, D. W.  
1939 Modern cereal chemistry (3rd ed.). Northern Publishing Co., Liverpool.
- Melville, J., and Shattock, H. T.  
1938 The action of ascorbic acid as a bread improver. *Cereal Chem.* **15**: 201-205.

- Morgan, E. J., and Friedmann, E.  
1938 Interaction of maleic acid with thiol compounds. *Biochem. J.* **32**: 733-742.  
1938a Maleic acid as inhibitor of enzyme reactions induced by thiol compounds. *Biochem. J.* **32**: 862-870.
- Potel, P., and Chaminade, R.  
1935 Sur le potentiel d'oxydo-réduction des farines. *Compt. rend.* **200**: 2215-2217.
- Read, J. W., and Haas, L. W.  
1938 Studies on the baking quality of flour as affected by certain enzyme actions. V. *Cereal Chem.* **15**: 59-68.
- Ritter, K.  
1939 Der Aufbau der Eiweisskörper und die Eiweisstoffe des Weizenmehls. *Mühlenlab.* **9**: 1-12.
- Schoen, M.  
1938 Faits nouveaux et nouvelles hypothèses dans la chimie des fermentations. *Ann. fermentations.* **4**: 257-283.
- Sullivan, B., and Howe, M.  
1937 The isolation of glutathione from wheat germ. *J. Am. Chem. Soc.* **59**: 2742.
- Sullivan, B., Howe, M., and Schmalz, M. D.  
1936a On the presence of glutathione in wheat germ. *Cereal Chem.* **13**: 665-669.
- Sullivan, B., Near, C., and Foley, G. H.  
1936 The harmful action of wheat germ on the baking quality of flour and the constituents responsible for this effect. *Cereal Chem.* **13**: 453-462.
- Treadwell, F. P.  
1927 *Kurzes Lehrbuch der analytischen Chemie.* Bd. 2, p. 557.
- Van Laer, M. H.  
1935 *La chimie des fermentations.* Masson et Cie., Paris.  
1935a Le potentiel d'oxydo-réduction au cours de la fermentation panaire. *Ann. fermentations* **1**: 402-405.

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## POLARISCOPIC DETERMINATION OF PROTEOLYTIC ACTIVITY

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(Read at the Annual Meeting, May 1939)

Both physical and chemical methods have been used extensively in determining proteolytic activity. Generally speaking, the physical methods are more sensitive than chemical methods. The rupture of a relatively small number of peptide bonds, for example, may change the physical property of the protein to a marked extent. It is well known that gelatin is a protein which is particularly susceptible to such changes. Concurrently with the setting of gelatin other properties show equally wide changes. The optical rotatory power of gelatin is one which is no exception.

Smith (1919) determined the polarization of gelatin as a function of temperature and found that there was a very large change between 10° and 25°.

Gore (1929) showed that this large change was destroyed when the

gelatin was digested with proteolytic enzymes, and furthermore that there was a regular relationship between the activity of the enzyme and the change in polarization.

### Principles of the Method

The marked effect on the polarization of gelatin as shown in Figure 1 can be utilized as a method of determining proteolytic activity. Experimentally it has been found that about 2% is the greatest concentration that can conveniently be used, because the solutions may

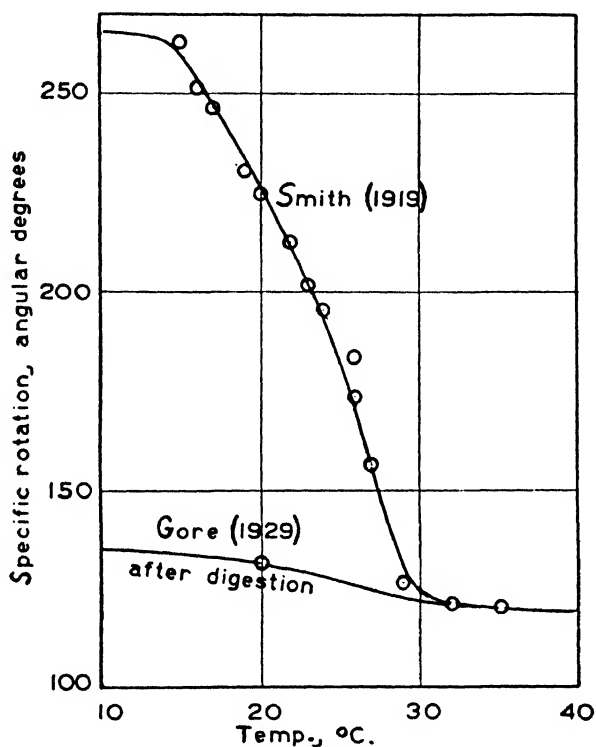


Fig. 1. Polarization of gelatin.

become too viscous to handle with greater concentrations. Probably the best method of stopping the reaction is by chilling. If the flask containing the reaction mixture is vigorously rotated in ice water, not more than one to two minutes is required to reduce the reaction velocity to one-tenth of its value at, say, 40°. The reaction may also be stopped by heating. Experimentally it has been found that the conversion occurring during the time required to raise the temperature from 40° to 80° by placing the flask in a boiling water bath cannot be detected in comparison with that occurring during an extended digestion period. Some malts must be treated in this matter in order to obtain a clear filtrate. Gore (1929) originally used 45° for the digestion, but it was



found that over long periods of time a slight destruction of enzyme occurred.

In order to be able to measure low activities a relatively long period of digestion must be chosen. Five hours has been tentatively considered as a convenient time. After digestion it would be preferable to read the polarization at, say,  $15^{\circ}$  or lower. Unless, however, one has a suitably conditioned room, fogging of the end glasses makes it almost impossible to read successfully at that temperature. Consequently  $20^{\circ}$  has been chosen as a convenient polarization temperature. As shown in Figure 1 the specific rotation changes over a 40% range.

### Standardization

It has been shown that one of the most satisfactory methods of standardizing an empirical procedure such as this is to relate the activity to the initial rates of conversion (Johnston and Jozsa, 1935).

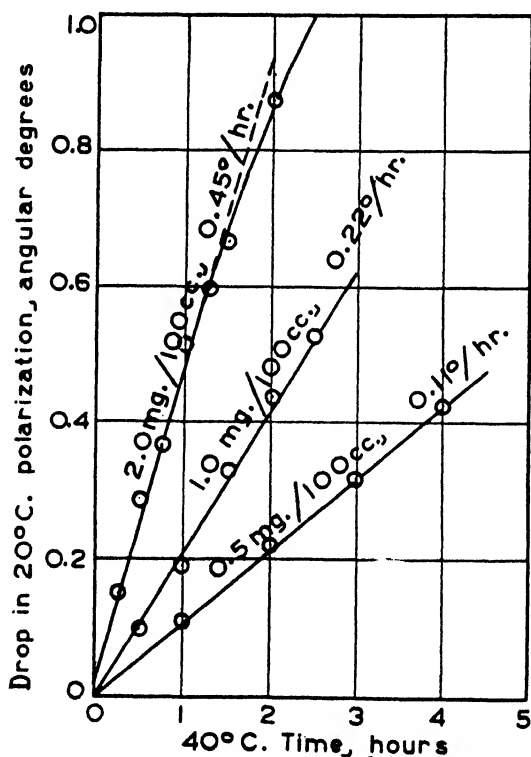


Fig. 2. Initial rates (standardization).

In this particular case it was desired to relate the activities observed to a scale which had previously been in use. A sample of known activity on this scale, namely, 25,000 milliunits per gram, was run at various concentrations. The curves in Figure 2 show respectively the

rates of conversion when the infusion contained 50, 25, and 12.5 milli-units of enzyme per 100 cc. of solution. The data lead to an average value of  $0.0089^\circ$  polarization change per hour per milliunit per 100 cc.

### Calibration and Procedure

The adopted details of the method may be a compromise between a number of factors which need not be listed for present purposes. A sample of a commercial food gelatin containing 10% moisture, 1% ash, and having a pH of 4.5 is used as substrate. This is made up to 2 g. of gelatin in 100 g. of solution, and buffered to a pH of 4.8 with acetate buffer. It is advisable to soak the gelatin in cold water before warming to  $40^\circ$ . Fresh substrate should be prepared for each day's run and toluene is used to prevent growth of organisms. To a 25-cc. portion of this substrate is added 25 cc. of enzyme infusion prepared in 2.5% sodium chloride solution, both at  $40^\circ$ , using 50-cc. stoppered Erlen-

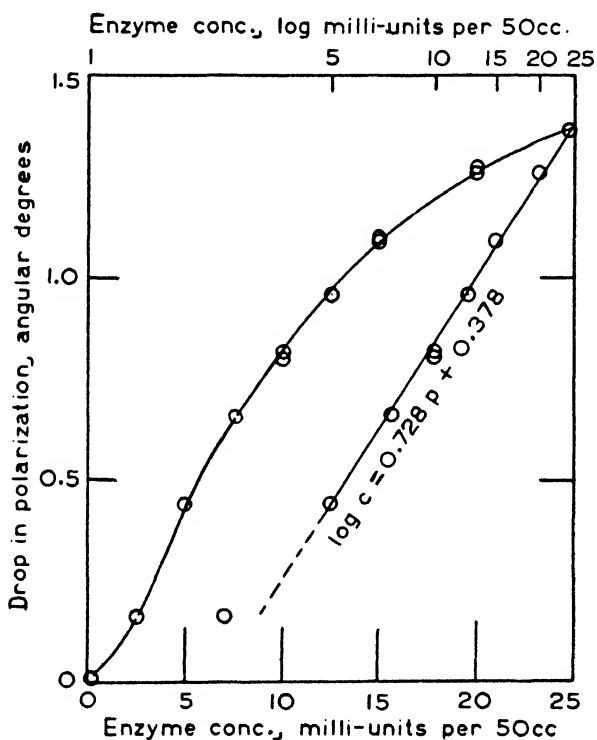


Fig. 3. Calibration.

meyers. After digestion for five hours the flasks are cooled rapidly in ice water and allowed to refrigerate for at least one hour. Alternatively, when excessively turbid enzyme extracts are used the reaction flask is placed in a vigorously boiling water bath for a few minutes until the "break" occurs, *i.e.*, until the soluble protein coagulates and flocc-

culates. The flask is then cooled and allowed to refrigerate for one hour. It is then placed in a 20° bath and polarized when equilibrium is attained. Usually this requires less than one hour. Filtration of turbid conversions can conveniently be made at 20°, but time must be allowed to attain equilibrium after filtration in case the solution has warmed up during the process. Both non-gelatin and non-enzymic blanks are run concurrently. The non-enzymic blank must be poured into a polariscope tube within one minute after cooling or it will gel. Using the sample from which the curves in Figure 2 were made, the data represented in Figure 3 were obtained. Over a range from approximately 10 to 25 milliunits per 50 cc. of reaction mixture the activity can be calculated by using the formula. At lower activities this function is invalid, but an estimate could be obtained by reading the data directly from the curve. A calibration performed three months after the one shown in Figure 3 with the procedure slightly modified for excessive summer temperatures gave  $\log c = 0.693p + 0.384$ , which is satisfactory agreement for this type of procedure. Some typical results are given in Table I. The procedure lends itself

TABLE I

Material	Concentration	Activity
	<i>gm. per 25 cc. infusion</i>	<i>M.U./gm.</i>
Papain No. 1	0.00075	24000
Papain No. 1 activated with H <sub>2</sub> S	0.00020	104000
Papain No. 2	0.00050	58000
Brewer's malt No. 1	2.0	4.1
Brewer's malt No. 2	2.0	3.6
Distiller's malt	2.0	6.1
Experimental malt No. 1	2.0	7.7
Experimental malt No. 2	2.5	1.3

readily to studies of activation. Normal flours are too low in activity to be tested by this method.

### Summary

The specific rotation of gelatin at temperatures below 25° is reduced by enzymic digestion. An empirical procedure relating the extent of the decrease to the concentration of enzyme is described.

### Literature Cited

- Gore, H. C.  
 1929 Action of papain on the polarization of gelatin. *Ind. Eng. Chem. (Anal. Ed.)* **1**: 203-205.
- Johnston, W. R., and Jozsa, S.  
 1935 A general method for determining the concentration of enzyme preparation. *J. Am. Chem. Soc.* **57**: 701-706.
- Smith, C. R.  
 1919 Mutarotation of gelatin and its significance in gelation. *J. Am. Chem. Soc.* **41**: 135-150.

# THE NUTRITIVE VALUE OF THE PROTEINS OF RICE AND ITS BY-PRODUCTS.<sup>1</sup> II. EFFECT OF AMINO ACID ADDITIONS ON GROWTH

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(Received for publication February 28, 1940)

In a previous communication (Kik, 1939) the results were reported of a study of the biological value of the proteins of rice and its by-products, and it was found that at a 5% protein level, the proteins of whole rice and those of polished rice had a lower biological value and a higher digestibility than those of rice bran and of rice polishings.

Digestibility and amino acid content of the food proteins are limitations of their nutritive value, and since both whole-rice and polished-rice proteins showed a high digestibility, it was believed necessary to study the effect of amino acid additions.

A study of the literature revealed that amino acid deficiencies have been found in garden peas (both fresh and canned), potatoes, beef, wheat, corn, and oats (Beadles, Braman, and Mitchell, 1930; Mitchell and Smuts, 1932), and hegari (Smith and Roehm, 1937).

This paper deals with a study of the effect on growth in rats fed rice diets with and without certain amino acid additions. The composition of the rations used in these experiments is given in Table I.

TABLE I  
COMPOSITION OF THE RATIONS

Ration	N	Salt mixture	Butter- fat	Starch
	%	%	%	%
Whole rice (88.0%)	1.05	4.0	8.0	—
Polished rice (88.0%)	0.92	4.0	8.0	—
Rice bran (67.0%)	1.32	4.0	8.0	21.0
Rice polishings (65.0%)	1.32	4.0	8.0	23.0

The amino acids, cystine, tyrosine, tryptophane, and the "leucine fraction" (a mixture of leucine, isoleucine, and valine) were prepared in the laboratory according to standard methods (Cole, 1933) and methionine and lysine were obtained from a reliable source.<sup>2</sup>

<sup>1</sup> Research paper No. 668, Journal Series, University of Arkansas.

<sup>2</sup> Amino Acid Manufacturers, Chemistry Department, University of California, Los Angeles, California.

TABLE II

AVERAGE EFFECT ON GROWTH OF THE ADDITION OF CYSTINE, METHIONINE, LYSINE, GELATIN, TRYPTOPHANE, AND "LEUCINE FRACTION" TO THE PROTEINS OF WHOLE RICE (6.0%), POLISHED RICE (5.5%), RICE BRAN (8%), AND RICE POLISHINGS (8.0%) AS SHOWN BY PAIRED-FEEDING EXPERIMENTS

Se- ries	No. prs.	Ration <sup>1</sup>	Ini- tial wt.	Fi- nal wt.	Gain	Duration of experiments	Food in- take	Supple- mental effect	P <sup>2</sup>
			g.	g.	g.	days	g.		
1	12	WR WR+cystine	61 61	125 130	5.0	84 for prs. 1-9 63 for prs. 10-12	606	+	.00018
2	6	WR WR+methionine	47 47	146 153	7.0	90	883	+	.00180
3	3	WR WR+tryptophane	42 42	125 125	None	100	704	None	—
4	6	WR WR+gelatin	53 53	140 144	4.0	44 for prs. 1-3 57 for prs. 4-6	927	+	.00270
5	3	WR WR+lysine	53 53	164 171	7.0	63	988	+	—
6	12	PR PR+cystine	49 49	107 111	4.0	105 for prs. 1-9 63 for prs. 10-12	698	+	.00271
7	6	PR PR+methionine	52 52	123 130	7.0	63	606	+	.00010
8	3	PR PR+tryptophane	41 41	101 99	-2.0	63	474	None	—
9	3	PR PR+gelatin	45 45	93 102	9.0	66	487	+	.003
10	3	PR PR+lysine	52	114 125	11.0	63	557	+	.0034
11	6	PR PR+leucine fraction	51	105 106	1.0	112	682	None	.0581
12	3	RB RB+cystine	46	175 174	-1	48	644	None	—
13	3	RP RP+cystine	47	177 177	None	61	659	None	—

<sup>1</sup> WR = whole rice. PR = polished rice. RB = rice bran. RP = rice polishings.

<sup>2</sup> The probability P is obtained according to Student's method (1908) and calculated from the differences between the average gains obtained. It is a criterion of high significance if smaller than .03.

### Plan of Experiment and Results

The paired-feeding method of Beadles, Braman, and Mitchell (1930) was employed; by this method the same amount of feed is supplied to both rats of any one group. The control animals of each litter-mate group received an additional 25 mg. of amino acid daily.

The animals were confined in individual cages and the feed and animals were weighed twice weekly. All animals received daily an ample supply of vitamin B consisting of 3 cc. of an aqueous extract of 5% brewer's yeast, 1 cc. of a riboflavin concentrate corresponding to 10 micrograms, 1 cc. of an extract of vitamin B<sub>1</sub> crystals (Merck) furnishing 20 micrograms, and 4 drops of cod liver oil as an additional source of vitamins A and D. Only male rats were employed of an initial weight of about 50 g.

*Whole rice.*—This was investigated in five series of paired-feeding experiments, in which additions were made of cystine, methionine, tryptophane, gelatin, and lysine. The results are summarized in Table II, which shows the number of pairs employed in each series, the average initial and final weights of the rats, the food intake, duration of each series, and the average differences between the gains in weight. A statistically significant supplemental effect was obtained when additions were made of cystine, methionine, gelatin, and lysine.

*Polished rice.*—In six series of experiments, additions were made of cystine, methionine, tryptophane, gelatin, lysine, and "leucine fraction," and statistically significant results were found for the amino acids, cystine, methionine, and lysine. Gelatin, a poor protein rich in lysine, had a supplementary effect.

*Rice polishings and rice bran.*—Three pairs of rats were employed in each series and cystine was added to the ration of the pair mates. No beneficial effect on growth was observed.

### Summary

Paired-feeding experiments showed that cystine, methionine, and lysine supplemented the proteins of whole rice and of polished rice to a slight but statistically significant extent. Tryptophane, however, did not have any beneficial effect.

Cystine did not promote growth as a supplement to the proteins of rice bran and rice polishings.

### Literature Cited

- Beadles, J. R., Braman, W. W., and Mitchell, H. H.  
1930 The cystine deficiency of the proteins of garden peas and of potatoes. *J. Biol. Chem.* **88**: 615-622.
- Cole, L. W.  
1933 *Practical physiological chemistry* (9th ed.). W. Heffer & Sons, Ltd., Cambridge.
- Kik, M. C.  
1939 The biological value of the proteins of rice and its by-products. *Cereal Chem.* **16**: 441-447.
- Mitchell, H. H., and Smuts, D. B.  
1932 The amino acid deficiencies of beef, wheat, corn, oats, and the soy beans for growth in the white rat. *J. Biol. Chem.* **95**: 263-281.

Smith, M. C., and Roehm, G. H.

1937 The biological value of the proteins in hegari and the supplemental value of certain protein concentrates used in farm animal feeding. *J. Agr. Research* 54: 135-145.

Student

1908 The probable error of a mean. *Biometrika* 6: 1-25.

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## EVIDENCE IN SUPPORT OF A RECENT PAPER CONCERNING THE EFFECT OF MILK ON THE BROMATE REQUIREMENTS OF FLOURS

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(Received for publication February 10, 1940)

Recently Ofelt and Larmour (1940) showed that in general the addition of milk solids to a modified "basic" formula increased the amount of potassium bromate necessary to produce a loaf of maximum volume by the conventional basic procedure. Their modification of the "basic" formula of the American Association of Cereal Chemists consisted of decreasing the yeast from 3% to 2%, increasing the sugar from 2.5% to 6%, increasing the salt from 1% to 1.75%, and superimposing 3% shortening and 6% powdered skim milk. A series of baking tests was made first with the indicated formula and second with the identical formula less the milk powder—with potassium bromate as a common variable ingredient. We quote from their summary:

In general the inclusion of 6% dry-milk solids creates a tolerance towards bromate which tends to prevent damage to loaf volume and to grain and texture when large dosages of this reagent are used. Even when the effect is small for loaf volume it remains marked for grain and texture. This buffering effect towards bromate has important commercial significance because it provides a safeguard against the possibility of damaging flours that have already been brought close to their optimum "oxidation" condition by bleaching or the addition of other oxidizing agents.

In view of the importance of this finding it seems worth while to present certain incidental data in support of their work. As in the study of Ofelt and Larmour, our baking procedure was a modification of the standard method approved by the American Association of Cereal Chemists. One hundred grams of flour (15% moisture basis) and the following percentage ingredients based on flour weight were employed: distilled water 60% (1% additional for each 1% of powdered skim milk), 3% yeast (type A as classified in a recent article by this writer, 1940), 2½% sucrose, 1% salt, 3% shortening (hydrogenated cottonseed) when indicated and 6% powdered skim milk when indicated. For the purpose of this paper we will consider the above a

"commercial" formula and when neither milk nor shortening is used the formula will be considered a "basic" formula. Unfortunately the data which we are about to consider were not acquired with the present purpose in mind and hence suffer a certain degree of incompleteness. Nonetheless, as can be seen from Table I, particularly when comparing the results obtained via the "commercial" versus the "basic" formula, our results are in complete agreement with those of Ofelt and Larmour

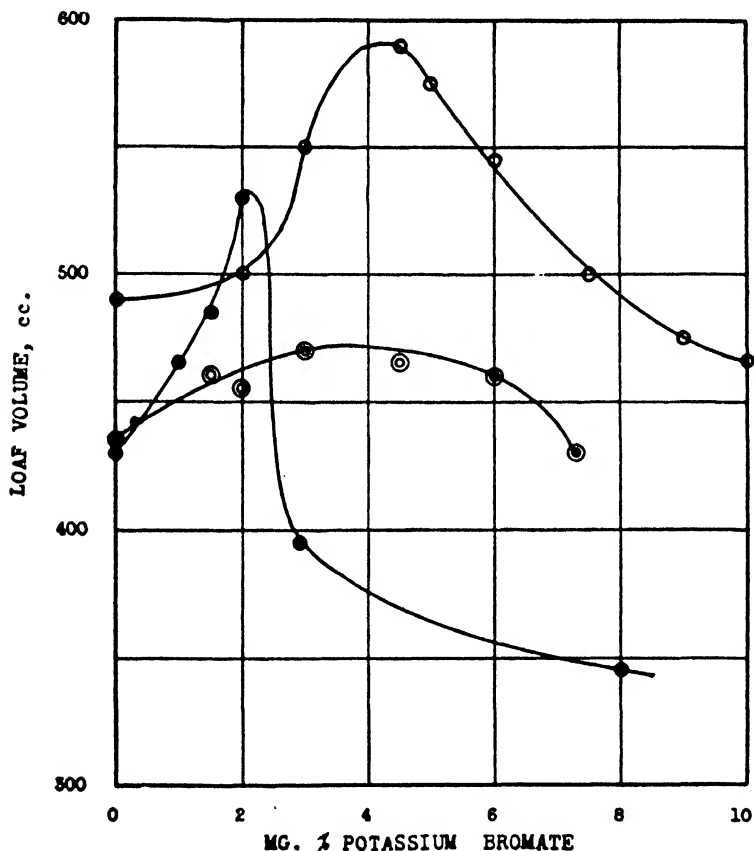


Fig. 1. Closed circles, "basic formula"; open circles, "commercial formula"; double circles, "basic formula plus 6% milk powder." The increased tolerance and response to potassium bromate in the presence of milk and shortening is evident as is the increased tolerance but decreased response to powdered skim milk alone.

—that is, if one may assume that the "basic" and the "basic plus shortening" formulas respond similarly to increments of potassium bromate. This assumption is justified in part by the two figures for shortening plus potassium bromate in Table I and in part by the resemblance of the "basic" and the "commercial" loaf-volume versus percentage potassium bromate curves in Figure 1 to certain of the corresponding figures in the paper under consideration.



TABLE I

LOAF VOLUME AS A FUNCTION OF FORMULA AND % POTASSIUM BROMATE

Bromate, mg. %	0.0	1.0	1.5	2.0	3.0	4.5	5.0	6.0	7.5	8.0	9.0	10.0
Basic	430	465	485	530	395	—	—	—	—	345	—	—
6% milk powder	435	—	460	455	470	465	—	460	430	—	—	—
Shortening, 3%	445	—	—	540	—	—	—	—	—	—	—	—
Commercial	490	—	—	500	550	590	575	545	500	—	475	465

On studying Table I in its entirety, however, one finds that the increased response and tolerance to potassium bromate in the presence of milk powder and shortening cannot be attributed to the milk powder alone. The use of only milk powder increases the tolerance to potassium bromate tremendously in that 0.0075% potassium bromate produced a loaf of volume identical to that produced in the absence of this salt, whereas in the "basic" formula this quantity of potassium bromate stimulated a distinct negative response. Milk powder itself, though apparently increasing the tolerance, actually decreases the response to potassium bromate.

Within these limitations, Figure 1 constitutes a remarkable corroboration of the figure on page 7 of Ofelt and Larmour's paper insofar as the "commercial" and the "basic" formulas are concerned. Where loaf volume versus potassium bromate for the case of "milk powder" versus the "basic" formula is indicated (double circles versus closed circles, Figure 1), a somewhat different interpretation of the bromate-milk effect may obtain. The problem of potassium bromate is indeed complex and these results may prove difficult to interpret on a basis of enzyme kinetics alone. Shortening, from these meager data, appears to play a supporting role in bromate action—at least in the presence of milk solids, if not in their absence.

Several questions of interest in addition are suggested by Figure 1. For example, with the yeast and flour (one very likely milled from Inter-mountain winter wheat and of 13.0% protein and of 0.41% ash) under consideration, a volume response of 120 cc. is shown merely by the addition of 3% shortening to a formula containing 6% milk powder and 0.004% potassium bromate. Further work in this direction may possibly lead to the development of a baking test of high sensitivity to shortening characteristics. Secondly, if, as may be postulated for the moment, "basic" and "basic plus shortening" loaf volumes are similar functions of potassium bromate content, then a volume response of the order of 215 cc. may be expected by superimposing 6% milk powder upon a formula containing 3% shortening and 0.004% potassium bromate. Hence a method of unusual sensitivity to milk charac-

teristics is indicated. Thirdly, the volume response inspired by milk powder and shortening as ingredients supplementary to the "basic" formula appears to be a rather critical function of the quantity of potassium bromate present. It may in fact be negative for certain percentages of potassium bromate and be positive for either larger or smaller quantities. If, therefore, as is already accepted, the volume response to potassium bromate is at least a rough measure of the "age" or past bleaching treatment of a flour, then we may expect either negative or positive volume responses to the addition of milk powder and shortening to random flour samples. And lastly, since our selection of 6% milk powder and 3% shortening as quantities suitable for study was entirely fortuitous insofar as this subject is concerned, another promising field of work is hereby predicted—that is, the problem of determining the relative interactions of shortening and milk powder with the view of discovering whether or not a relationship exists between the optimum quantity of the one as a function of any selected quantity of the other.

These four suggestions have engaged this laboratory's attention for some time past, but unfortunately other interests have prevented and apparently will continue to prevent their study here. We therefore pass them on with the hope that other workers in a better position than we to attempt the necessary pure research will carry on. Certainly a new key to the study of the bromate effect lies here.

### Summary

This writer agrees with the opinion of Ofelt and Larmour that their discovery of the "buffering" effect of milk powder to increments of potassium bromate is one of prime importance. Corroborative evidence is presented. A suggestion with supporting evidence that shortening is deeply involved in the effect is made.

### Literature Cited

Ofelt, C. W., and Larmour, R. K.

1940 The effect of milk on the bromate requirements of flours. *Cereal Chemistry* 17: 1-18.

Eisenberg, S.

1940 Gas production in yeast fermentation and its applications. II. A volumetric method for the determination of gas production. *Cereal Chemistry* 17: 430-447.

# QUALITY STUDIES ON NORTH DAKOTA DURUM WHEATS (1938 CROP)

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(Received for publication February 1, 1940)

The principal area of durum wheat production in the United States lies in the north-central section of the state of North Dakota. In this region the environmental conditions of soil and climate are conducive to the production of high-quality durum for macaroni manufacture. Despite the importance of this grain crop to North Dakota, until quite recently no satisfactory facilities for making quality studies on durum wheats in line with commercial procedures have been available. This fact was due principally to a lack of published information regarding the experimental processing of macaroni. Macaroni products of various types had been grouped together under the general designation of "alimentary pastes," under such names as macaroni, spaghetti, vermicelli, etc., to describe specific products. LeClerc (1933) proposed the adoption of the term "macaroni" or "macaroni products" as a general designation for this class of food products. When a specific physical form is referred to, it is indicated by the conventional name.

Fifield (1934) and Fifield *et al.* (1937) discussed experimental apparatus and methods for the manufacture of macaroni products and reported the results obtained upon durum wheats grown in the hard red spring wheat region of the United States from 1932 to 1936. The accumulated data presented showed that inherent quality differences exist between varieties, and that these differences are surprisingly consistent over wide ranges of seasons and environments.

Binnington and Geddes (1936) have described in detail experimental milling and processing equipment for durum wheats, based upon commercial procedures. Their article also contains a critical discussion of the methods employed in standardizing the various operations involved. Several photographs of equipment are included in their paper. The effects of variations in absorption, kneading time, and rest period and the effect of press temperature upon color characteristics, as measured by a spectrophotometer, were investigated and these variables standardized to produce optimum results in terms of color analysis. A statistical basis for establishing criteria of the differences required for distinguishing between different samples is presented. Binnington and Geddes (1937) examined 34 samples of experimentally grown durum wheats produced in Canada during 1934 and 1935, using the standardized methods previously developed. Color

analyses were made and a single-figure color estimate developed, which corresponded satisfactorily with visual color score. Quality differentiation, in terms of color and appearance, was demonstrated between the samples. Little relationship appeared to exist between the carotenoid content of durum wheat and the color of the macaroni product derived from it.

Borasio (1935) published a report dealing with the determination of the cooking quality of alimentary pastes and described methods developed for their investigation. This work was reviewed in some detail by Binnington, Johansson, and Geddes (1939) and Harris and Knowles (1939). Borasio listed the principal factors of interest from the cooking standpoint as: (1) degree of cooking required, (2) resistance to disintegration, (3) capacity for absorption of water, and (4) increase in the volume of the paste.

C. E. Mangels and E. Latzke (unpublished data, North Dakota Agricultural Experiment Station, 1934) attempted to develop a method for determining quantitatively the cooking quality of macaroni products. These workers measured the increases in length and diameter of 7-centimeter lengths of macaroni after cooking. The increase in weight during cooking was ascertained, as well as the degree of disintegration of the sample.

An additional test of cooking quality was developed by Binnington, Johansson, and Geddes (1939) for measuring the tenderness of cooked macaroni. These workers constructed an apparatus modeled after the instrument described by Bonney, Clifford, and Lepper (1931) for testing the tenderness of canned fruits and vegetables, but containing some additional features. A full description of this equipment, including a photograph and plan of construction, is included in their paper. Refined methods for determining dry volume, water absorption, increase in volume, and degree of disintegration upon cooking are also given.

Further studies of the relative macaroni-making qualities of a number of durum wheat varieties were published by Binnington and Geddes (1939). From the results of these studies, the authors emphasized the point that macaroni quality cannot as yet be predicted from a single analytical test applied to the wheat and that wheat carotene is valueless as an index of macaroni color, particularly for intervarietal prediction.

### Experimental Material and Methods

Seventeen samples<sup>1</sup> of various varieties of durum wheat grown at Fargo and Langdon, North Dakota, in the 1938 crop year, were ex-

<sup>1</sup> These samples were supplied by T. E. Stoa, Chairman of Agronomy, North Dakota Agricultural Experiment Station, and Glenn Smith, Associate Agronomist, United States Department of Agriculture.

perimentally milled and the resultant semolinas processed into macaroni, using the standardized techniques developed by Binnington and Geddes<sup>2</sup> (1936). Langdon is situated in the area noted for durum wheat production, while Fargo is south of this area. Color analyses were also made on the macaroni, as well as various other analytical tests. Determinations of weight, volume, and tenderness of the cooked macaroni, as well as the amount of disintegration, were made at the North Dakota Experiment Station, using the methods described by Binnington, Johansson, and Geddes.

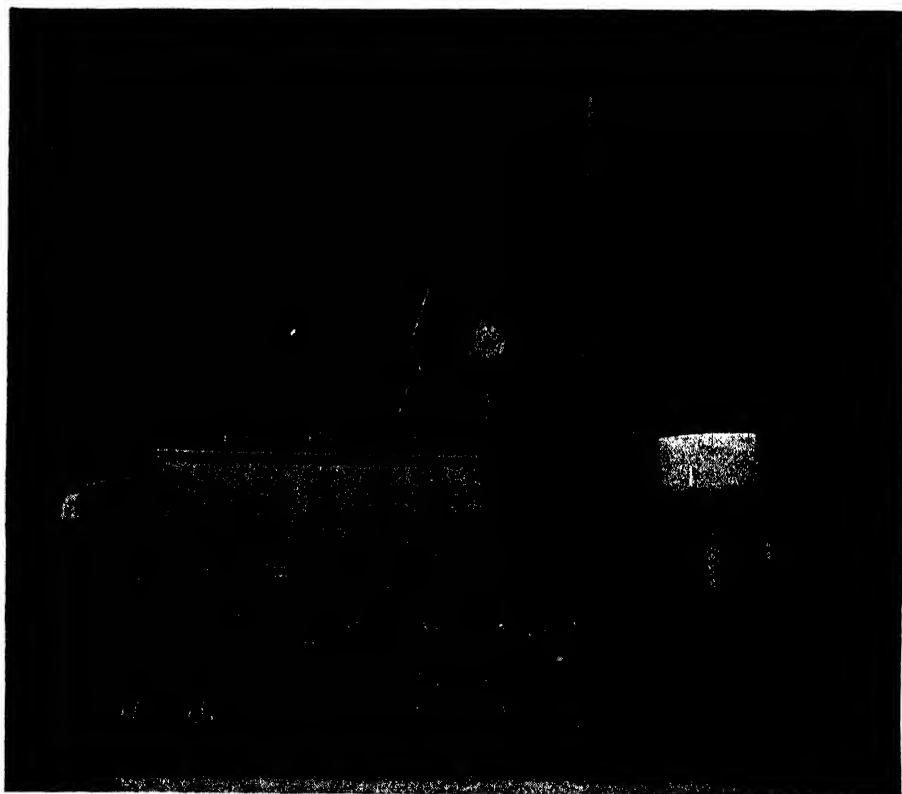


Fig. 1. *A*—Constant-temperature macaroni cooker. *B*—Volumeter used for measuring the increase in volume after cooking. *C*—Volumeter used for measuring the dry volume before cooking. *D*—High-form lipless 500 cc. Pyrex beakers used as containers for the macaroni during cooking.

The macaroni products were cooked in a constant-temperature bath similar to the cooker used by the Canadian laboratory. Prestone was used in the bath rather than water, because of the relatively high temperature employed. A thin layer of mineral oil was added to the Prestone to minimize the evaporation. The temperature was controlled within  $\pm 0.5^{\circ}\text{C}$ . Six samples were run at one time. A photograph of the apparatus and accessory equipment is shown in Figure 1.

<sup>2</sup> This phase of the experimental work, as well as the analytical tests and color determinations, was carried out by the Dominion Grain Research Laboratory, Winnipeg, Canada, to whom the authors' thanks are due.

The method of procedure was as follows: 500-cc. tall-form lipless beakers were placed in the bath, and 250 cc. of distilled water, previously heated to  $95^{\circ}\text{C}.$ , was added to each beaker. When the temperature of the water in the beaker reached  $95.5^{\circ}\text{--}96^{\circ}\text{C}.$ , 25 g. of the macaroni product was introduced into the beakers containing the water. The samples were cooked for 30 minutes and stirred at ten-minute intervals throughout the cooking period. Binnington, Johannson and Geddes (1939) had previously established that the 30-minute period was the optimum time of cooking. Further cooking results in excessive softening. At the end of the cooking period the macaroni was drained on a Büchner funnel and washed with boiling distilled water. At the end of a 10-minute draining period the macaroni was transferred to a watch glass and weighed.

The increase in volume was determined by the method described by Binnington, Johannson, and Geddes (1939).

The amount of residue was determined by evaporating the entire amount of the cooking water and washings to dryness and weighing. Previous investigators used aliquots of the cooking water, but, because of the colloidal nature of the liquid and attendant difficulties of securing a representative aliquot, evaporation of the entire quantity seemed expedient.

The method used for determining the tenderness of the cooked macaroni corresponded in essential details with that described by Binnington, Johannson, and Geddes (1939). Load is applied to the sample at a constant rate of approximately 12 g. of mercury per second from a constant head device. The orifice of the delivery tube was adjusted to deliver 57 cc. per minute. The delivery rate was checked quite frequently, as it tends to decrease with surface oxidation. A record was made upon a kymograph drum driven by a small electric motor. A typical record obtained by the use of this instrument and a plan and photograph of the apparatus are shown by Binnington, Johannson, and Geddes. In calculating the tenderness scores, the time required to compress the sample to a thickness of 0.135 inch was used instead of 0.115 inch, as used by those workers. It was necessary to use this value since the lower value of 0.115 did not fall on the linear portion of the curve in all cases. In view of this fact, the tenderness scores obtained are not comparable to the scores reported by Binnington, Johannson, and Geddes. The tenderness tester used in this study was constructed by D. S. Binnington, General Mills Research Laboratory, Minneapolis, Minnesota.

### Discussion

Description of the wheats used, semolina yield, and analytical data are shown in Table I. Nine of the samples were grown at Fargo and

TABLE I  
WHEAT DESCRIPTION, PROTEIN, ASH, AND PIGMENT DATA <sup>1</sup>

Sample No.	Variety	Semo- lina yield	Protein		Ash		Pigment	
			Wheat	Semo- lina	Wheat	Semo- lina	Wheat	Semo- lina
		%	%	%	%	%	ppm.	ppm.
FARGO								
39-5-13	Kubanka 75	36.6	11.6	10.2	1.63	0.58	5.72	4.53
39-5-1	Kubanka 49	37.5	11.7	10.0	1.74	0.58	6.33	5.35
39-5-11	Kubanka 1440	38.1	11.8	10.4	1.80	0.68	5.81	4.72
39-5-7	Mindum	39.9	12.2	10.8	1.60	0.53	5.78	4.60
39-5-17	Kubanka 314	38.4	12.3	11.1	1.75	0.58	6.31	5.18
39-5-4	Ld 26	38.1	12.5	11.3	1.76	0.57	3.61	2.62
39-5-16	Golden Ball	37.9	13.2	11.3	1.86	0.65	6.15	4.89
39-5-5	Ld 34	40.4	13.2	11.6	1.66	0.62	5.05	4.11
39-5-3	Monad	35.3	13.3	11.4	1.70	0.62	4.42	2.71
Average		38.02	12.42	10.9	1.72	0.60	5.46	4.30
LANGDON								
39-5-12	Ld 31	36.6	12.0	10.3	1.76	0.64	5.89	4.78
39-5-14	Mindum	36.2	13.0	11.7	1.62	0.69	7.12	5.64
39-5-10	Golden Ball	35.2	13.6	11.6	2.01	0.78	8.56	5.96
39-5-8	R. L. 1183	36.2	13.7	11.9	1.69	0.62	7.61	6.04
39-5-15	Ld 34	36.5	13.7	12.0	1.42	0.60	6.10	4.80
39-5-9	Kubanka 1440	32.5	13.8	12.6	1.83	0.68	7.58	3.65
39-5-2	Monad	33.8	14.0	12.5	1.80	0.63	5.87	3.73
39-5-6	Ld 9 X Mindum	38.1	14.1	12.5	1.72	0.58	6.63	4.67
Average		35.64	13.49	11.89	1.73	0.65	6.92	4.91

<sup>1</sup> Analytical results reported on a 13.5% moisture basis.

eight at Langdon. A number of varieties were included, according to their known characteristics, to cover the range from excellent to poor in macaroni quality. A substantial variation in protein and pigment content is evident among the samples. Although these samples have been arranged in order of increasing wheat protein content, it must be borne in mind that protein content has not been proved to be an important factor in ranking durum wheats for quality, as is the case with bread wheats. The semolina yield, of course, is lower than would have been the case if flour had been produced instead. It will be noticed that the Fargo wheats produced more semolina than the Langdon ones, but were lower in wheat and semolina protein and pigment content. The fact that Langdon durums are higher in pigment content than Fargo durums is in line with evidence already accumulated at this Experiment Station from previous tests.

Table II shows comparative color analyses of the semolina and macaroni produced from the wheats. These values are listed under

TABLE II  
COLOR ANALYSES OF SEMOLINA<sup>1</sup> AND MACARONI<sup>2</sup> MADE FROM VARIETIES OF  
NORTH DAKOTA DURUM WHEAT (1938 CROP)  
(Arranged in order of increasing macaroni color score.)

Sample No.	Variety	Hue		Saturation		Brilliance		Single-figure color score	
		Semo-lina	Macaroni	Semo-lina	Macaroni	Semo-lina	Macaroni	Semo-lina	Macaroni
FARGO									
39-5-4	Ld 26	24.70	23.34	5.87	5.04	9.09	7.06	16.0	16.7
39-5-16	Golden Ball	24.77	22.72	3.80	5.22	8.83	6.84	10.7	17.3
39-5-11	Kubanka 1440	24.70	23.09	4.19	5.44	8.86	7.03	11.7	17.9
39-5-3	Monad	24.68	22.34	4.55	5.52	8.84	6.82	12.7	18.1
39-5-1	Kubanka 49	24.70	23.32	4.12	5.88	9.02	7.26	11.3	18.9
39-5-7	Mindum	24.77	23.54	4.27	5.96	8.92	7.30	11.9	19.2
39-5-17	Kubanka 314	24.74	23.29	4.05	5.88	8.94	7.06	11.2	19.4
39-5-5	Ld 34	24.72	23.41	4.55	6.28	8.90	7.18	12.6	20.5
39-5-13	Kubanka 75	24.76	23.12	4.46	6.36	8.95	7.06	12.3	20.8
Average		24.73	23.13	4.43	5.73	8.93	7.07	12.3	18.8
LANGDON									
39-5-10	Golden Ball	24.70	22.42	3.38	5.68	8.74	6.70	9.6	19.0
39-5-2	Monad	24.66	22.38	3.82	5.74	8.73	6.59	10.8	19.5
39-5-9	Kubanka 1440	24.79	22.50	3.54	5.88	8.80	6.68	10.0	19.8
39-5-8	R.L. 1183	24.73	23.03	3.23	6.18	8.78	6.83	9.1	20.8
39-5-6	Ld 9 X Mindum	24.72	23.22	4.38	6.42	8.97	7.03	12.1	21.2
39-5-12	Ld 31	24.62	22.82	3.94	6.44	8.83	6.88	11.0	21.4
39-5-14	Mindum	24.74	22.93	3.46	6.42	8.80	6.89	9.7	21.4
39-5-15	Ld 34	24.77	23.06	4.37	6.82	8.93	7.01	12.1	22.4
Average		24.72	22.80	3.76	6.20	8.82	6.83	10.6	20.7

<sup>1</sup> Data secured with Munsell disks Y-YR 8/6; Y 8/12; N 8/; N/5. Unbalanced illumination N 9.4/; N 8/.

<sup>2</sup> Data secured with Munsell disks YR 6/12; Y 8/12; N 7/; N 4.

three sections: hue, saturation, and brilliance. Binnington and Geddes (1937, 1939) have formulated a color score by combining these three factors in the following manner:

$$\text{Color score} = \frac{\text{Hue}}{\text{Brilliance/Saturation}}$$

The values for hue, brilliance, and saturation were computed according to the formula outlined by Nickerson (1929) and the color score has been found by Binnington, Johannson, and Geddes to give results in close agreement with careful visual classification when working with varietal material. For a further discussion of the color problem reference is made to the papers cited.

It is apparent from the data presented that the Langdon wheats produced macaroni of better and more acceptable color than the Fargo samples. This result is in agreement with conclusions drawn from visual inspection of macaroni produced from wheats grown at these respective locations. The final four wheats in the table, Ld 34, Mindum, Ld 31, and Ld 9 X Mindum, were judged to be entirely satisfactory from the standpoint of color.



It is interesting to note that the color score of Monad grown at Langdon was higher than that of the Mindum grown at Fargo, although it is extremely doubtful if this difference is significant. The variety Ld 26 gave a macaroni having a very undesirable grayish color due, in part at least, to an abnormally low pigment content. Kubanka invariably gives a very pale macaroni, Monad a product possessing a reddish cast, and Golden Ball a macaroni of very unsatisfactory color.

In Table III the comparative cooking quality data obtained from

TABLE III  
COMPARATIVE COOKING-QUALITY DATA <sup>1</sup> OBTAINED ON MACARONI  
PROCESSED FROM THE 1938 CROP  
(Arranged in order of increasing weight and volume after cooking.) <sup>2</sup>

Sample No.	Variety	Cooked weight	Increase in weight	Cooked volume	Increase in volume	Residue	Tender-ness score
		g.	g.	cc.	cc.	%	
FARGO							
39-5-16	Golden Ball	350.3	250.3	322.0	252.0	4.5	101.18
39-5-5	Ld 34	354.1	254.1	327.6	257.6	4.8	119.12
39-5-7	Mindum	357.7	257.7	328.4	258.4	4.2	96.67
39-5-3	Monad	358.9	258.9	322.0	262.0	4.8	93.28
39-5-13	Kubanka 75	365.8	265.8	337.8	267.8	4.5	92.52
39-5-4	Ld 26	376.5	276.5	346.8	276.8	4.6	93.42
39-5-1	Kubanka 49	381.4	281.4	353.2	283.2	4.4	97.96
39-5-17	Kubanka 314	382.0	282.0	353.2	283.2	4.3	97.97
39-5-11	Kubanka 1440	382.4	282.4	352.8	282.8	4.0	90.57
Average		367.7	267.7	338.2	269.3	4.4	98.08
LANGDON							
39-5-9	Kubanka 1440	346.9	246.9	318.4	248.4	3.8	115.37
39-5-8	R. L. 1183	353.5	253.5	325.6	255.6	4.6	100.64
39-5-14	Mindum	355.2	255.2	328.8	258.8	4.5	107.65
39-5-10	Golden Ball	356.2	256.2	327.6	257.6	4.1	116.44
39-5-15	Ld 34	356.7	256.7	328.8	258.8	4.0	102.50
39-5-6	Ld 9 × Mindum	358.9	258.9	330.4	260.4	4.4	128.11
39-5-2	Monad	370.3	270.3	342.8	272.8	4.5	122.13
39-5-12	Ld 31	381.3	281.3	354.8	284.8	4.8	101.79
Average		359.9	259.9	332.2	262.2	4.3	111.83

<sup>1</sup> All results calculated on 100 g. of material containing 13.5% moisture.

<sup>2</sup> A constant dry volume of 70 cc. was obtained.

the macaroni after processing are shown. As the increase in weight and volume are a measurement of the water absorbed during the cooking process, these values are considered to be of primary importance in ranking macaroni for cooking quality.

The values for cooked weight, on the basis of 100 g. of dry material, varied from a minimum of 346.9 g. to a maximum of 382.4 g. These values are somewhat lower than the results reported by Binnington, Johansson, and Geddes (1939). There is a corresponding range in the volumes of the cooked macaroni from 318.4 cc. to 354.8 cc. These

values are also lower than the cooked volumes reported by the Canadian workers.

The average values for both weight and volume of the cooked macaroni samples in this series were higher than similar values of a series of commercial samples<sup>3</sup> reported by Harris and Knowles (1939). This would be expected, as the series of commercially manufactured macaroni contained samples made entirely, or in part, of farina.

A constant dry volume of 70 cc. was obtained on this series of samples. Binnington reported a range in dry volume of 69.6 cc. to 73.2 cc.

The results do not indicate any relationship between color and cooking quality. In a previous study of commercial macaroni the color ranking closely followed the order of cooking quality. This commercial series consisted of macaroni made from varying grades of semolina, mixtures of semolina and farina, and pure farina, and it was found that the macaroni made from the best semolina outranked the other samples both in color and in cooking quality.

The following four varieties appear to be the most satisfactory from the standpoint of cooking quality:

Kubanka	1440—Fargo
Kubanka	314—Fargo
Kubanka	49—Fargo
Ld	31—Langdon

In regard to the tenderness score of the samples, the Langdon series was significantly higher than the Fargo series.

The means, standard deviations, and coefficients of variability computed for the different variables are shown in Table IV, while the correlation coefficients calculated from the data are presented in Table V. It will be noticed that the relationship between cooked weight and cooked volume is very high in each series of samples and justifies the prediction of cooked volume from cooked weight by the following formula, when 25 g. of macaroni product are used:

$$\text{Cooked volume} = -11.34 + 1.0455 \times \text{cooked weight.}$$

$$\text{Error of estimate} = 0.55 \text{ cc.}$$

As the determination of cooked weight is more convenient, rapid, and precise than the determination of cooked volume, the former measurement appears to possess more utility than the determination of volume.

It is also evident that as the protein content increases the tenderness score likewise tends to increase, while the cooked weight tends to decrease. Previous studies at this station have shown that the cooking

<sup>3</sup> The samples were supplied through the courtesy of General Mills, Pillsbury Mills, and Dr. B. R. Jacobs, Director of Research, National Macaroni Manufacturers' Association.

TABLE IV  
TABLE OF STATISTICAL CONSTANTS—MEANS, STANDARD DEVIATIONS, AND  
COEFFICIENTS OF VARIABILITY

	Means	Standard deviations	Coefficients of variability
EXPERIMENTAL SAMPLES [N = 17]			
Cooked weight, g.	91.00	2.993	3.29
Cooked volume, cc.	83.98	2.944	3.50
Wheat protein, %	12.92	0.831	6.43
Semolina protein, %	11.36	0.793	6.98
Wheat ash, %	1.726	0.124	7.16
Semolina ash, %	0.637	0.084	13.16
Tenderness score	104.55	11.174	10.69
COMMERCIAL SAMPLES [N = 18]			
Cooked weight, g.	81.68	5.217	6.39
Cooked volume, cc.	73.89	5.332	7.22
COMBINED EXPERIMENTAL AND COMMERCIAL SAMPLES [N = 35]			
Cooked weight, g.	86.21	6.329	7.34
Cooked volume, cc.	78.80	6.641	8.43

TABLE V  
CORRELATION COEFFICIENTS COMPUTED FROM ANALYTICAL AND COOKING  
QUALITY TEST DATA

Variables correlated			
X	Y	$r_{xy}$	P <sup>1</sup>
EXPERIMENTAL SAMPLES [N = 17]			
Cooked weight, g.	Cooked volume, cc.	<b>+ .9970<sup>2</sup></b>	< .0001
Cooked weight, g.	Semolina protein, %	<b>- .6227</b>	.0087
Cooked weight, g.	Tenderness score	<b>- .4174</b>	.0768
Semolina protein, %	Tenderness score	<b>+ .7306</b>	.0009
Semolina protein, %	Wheat protein, %	<b>+ .9529</b>	< .0001
Wheat ash, %	Semolina ash, %	<b>+ .2901</b>	.2616
COMMERCIAL SAMPLES [N = 18]			
Cooked weight, g.	Cooked volume, cc.	<b>+ .9937</b>	< .0001
Cooked weight, g.	Macaroni protein, %	<b>- .7037</b>	.0011
COMBINED COMMERCIAL AND EXPERIMENTAL SAMPLES [N = 35]			
Cooked weight, g.	Cooked volume, cc.	<b>+ .9966</b>	< .0001

<sup>1</sup> Probability of the observed correlation coefficient arising from uncorrelated material through errors of random sampling.

<sup>2</sup> Significant correlation coefficients are shown in heavier type.

procedure apparently does not remove appreciable quantities of protein from macaroni, regardless of the type of wheat from which the macaroni is produced. Substantial proportions of the ash, however, were found to be soluble in the cooking water.

The work reported here will later be extended to a more comprehensive study of North Dakota durum wheats, embracing several years' crops. The present study can only be regarded as a preliminary investigation.

### Summary and Conclusions

Although a relatively small number of samples were included in the present study, the data obtained would appear to justify the following conclusions:

Durum wheats grown at the Fargo station were higher in semolina yield than wheats grown at Langdon, as a result of differences in test weights.

The Langdon wheats, however, were higher in protein content and produced macaroni of better and more acceptable color. Varietal color differences were also indicated. The "tenderness" score of these samples was also higher than that of the Fargo samples. The latter group of macaroni samples might possibly be classified as definitely "soft" for commercial purposes although the tenderness score limits for commercially acceptable macaroni have not yet been established. There was no relationship between color and cooking quality in this series of samples.

Inasmuch as the determination of cooked weight is more rapid and precise than the determination of cooked volume, the former value is to be preferred, especially as the correlation coefficient between the two variables is sufficiently high to predict the latter variable from the former with a low error of estimate. It was also found unnecessary to determine the dry volume of the macaroni, owing to the relatively constant value obtained for this property.

A negative relationship was evident between cooked weight and protein.

### Acknowledgment

The authors are greatly indebted to L. D. Sibbitt,<sup>4</sup> who conducted the milling, processing, and color determinations. Thanks are due to John H. Monge for his valuable technical assistance in obtaining the data presented in this paper.

### Literature Cited

- Binnington, D. S., and Geddes, W. F.  
1936 Experimental durum milling and macaroni-making technique. *Cereal Chem.* **13**: 497-521.  
1937 The relative macaroni-making quality of a number of durum wheat varieties. *Cereal Chem.* **14**: 293-304.  
1939 Further studies upon the relative macaroni-making quality of a number of durum wheat varieties. *Cereal Chem.* **16**: 384-392.  
Binnington, D. S., Johansson, H., and Geddes, W. F.  
1939 Quantitative methods for evaluating the quality of macaroni products. *Cereal Chem.* **16**: 149-167.  
Bonney, V. G., Clifford, P. A., and Lepper, H. A.  
1931 An apparatus for determining the tenderness of certain canned fruits and vegetables. *U. S. D. A. Circ.* **164**.  
Borasio, Luigi  
1935 Le caratteristiche di cottura della pasta ed. i metodi di ricerca. *Giorn. di Riscicoltura* **25**: 251-257.

<sup>4</sup> Formerly of the Dominion Grain Research Laboratory, Winnipeg, Canada. At present, Assistant Cereal Technologist, North Dakota Agricultural Experiment Station.

Fifield, C. C.

1934 Experimental equipment for the manufacture of alimentary pastes. *Cereal Chem.* 11: 330-334.

— Clark, J. A., Smith, G., Hayes, D. F., Christie, A., and Hoffecker, E.

1937 Milling and macaroni experiments with durum wheats. Mimeographed report, Bur. Plant Ind., U. S. D. A.

Harris, R. H., and Knowles, Darline

1939 The importance of durum wheat in American Agriculture and Industry. Part III. The determination of the quality of durum wheat and macaroni products. Experimental milling of semolina, macaroni manufacture, and evaluation of cooking quality. *The Macaroni Journal* 21: (No. 7) 6-8, 10, 12.

LeClerc, J. A.

1933 Macaroni products. *Cereal Chem.* 10: 383-419.

Nickerson, Dorothy

1929 A method for determining the color of agricultural products. U. S. D. A. Tech. Bul. 154.

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## THE EFFECT OF ETHYLENE ON FRESHLY HARVESTED WHEAT<sup>1</sup>

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(Received for publication January 10, 1940)

Changes commonly associated with ripening have been shown to occur in several agricultural products after subjecting them briefly to small concentrations of ethylene in air. The effect was first described by Denny (1924) on mature green lemons, where exposure to ethylene, even when diluted 1/1,000,000, resulted in a more active evolution of CO<sub>2</sub> from the fruit and the rapid appearance of yellow color in the skin. Chace and Church (1927) have demonstrated a similar effect on oranges, accompanied by disappearance of free acid and increase in sugar, and Chace and Sorber (1928) have shown that pears rapidly convert starch to sugar and undergo an apparently typical ripening. More recently the view that ethylene produces a natural rather than an artificial ripening has been strengthened by the observation that apples give off ethylene during the natural ripening process (Gane, 1934), which in turn acts on any neighboring green fruit. The effect of ethylene is not confined to fruits, for Chace and Sorber (1936) have shown that walnuts treated therewith are ripened to the extent that the hulls become loose.

In the harvesting of wheat two methods are in common use, the older method whereby wheat is harvested and allowed to remain in the field and the "combine" method, in which the wheat is threshed at the time of cutting and thereafter stored in bins. The after-ripening process sometimes designated as "sweating" is less likely to cause

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<sup>1</sup> Food Research Division Contribution No. 472.

trouble when the older method of harvesting is employed than when the wheat is combined. With the former method this biological change largely occurs while the wheat is standing in the shock, whereas with the latter it is delayed, and frequently causes trouble after the wheat has been milled into flour. Swanson (1935) reported that combined wheat is likely to be unsuitable for bread making until it has been aged for some weeks to afford opportunity for after-ripening changes to occur.

The following experiments were conducted with the thought that ethylene might hasten the after-ripening of combined wheat, possibly in such a manner that the grain might become more quickly available for use and the period during which "sweating" takes place might be considerably shortened.

### Experimental

Freshly harvested combined wheat (hard spring) was shipped directly from the field to the laboratory, under ice refrigeration. Immediately on arrival the grain, containing 17.1% moisture,<sup>2</sup> was cleaned and stored at  $-1^{\circ}$ . Two days later some of the grain was placed in air containing one part per thousand of ethylene and kept there at room temperature for three days, the air and ethylene being renewed daily. A similar portion of the grain was allowed to stand for three days at room temperature without any treatment. The two samples were then milled and the flour used in regular baking tests.<sup>3</sup> The bread from untreated wheat had smaller volume and was soggy and decidedly greenish in color. That from the ethylene-treated wheat was of excellent texture, color, and apparently in all respects normal (Fig. 1). The treated and untreated samples of the wheat were then both held at room temperature, and several times flour was made from a portion of each and baked as before. The appearance of the bread made after allowing various intervals to elapse between the treatment with ethylene and the milling showed that the untreated wheat gradually attained, after about a month, the characteristics shown after three days by the wheat exposed to ethylene.<sup>4</sup> The data in Table I illustrate this point. Unfortunately, it was not possible to

<sup>2</sup> 17.1% moisture is rather high for combined wheat. The wheat was fully ripe when harvested under moist conditions for experimental work and shipped to us under ice refrigeration. At room temperature, at which our experiments were carried out, the wheat immediately lost this excess moisture and had the appearance of a good hard spring wheat slightly bleached.

<sup>3</sup> Baking formula (standard loaves):

Flour.....	315 g.
Salt.....	5.5 g.
Yeast.....	8.0 g.
Sugar.....	20.0 g.
Shortening.....	6.5 g.
Water.....	170 cc.

<sup>4</sup> The improvement of wheat by treatment with ethylene had been previously demonstrated by us with some wheat we obtained through the Foreign Agricultural Service from Argentina during March, 1939. However, the wheat was several weeks old by the time we received it and the results of the experiments were not so striking. The flour from the ethylene-treated wheat made decidedly better bread than the controls of untreated wheat.

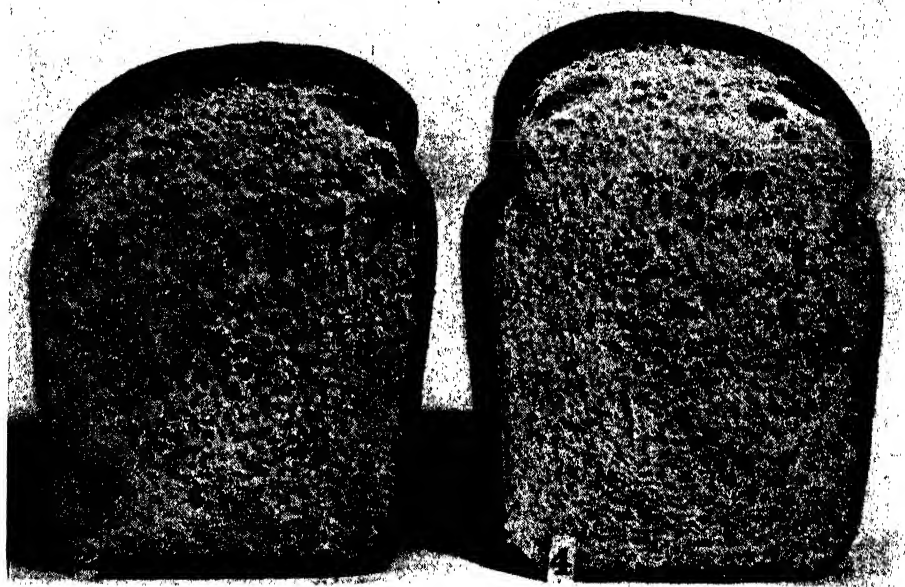


Fig. 1. Effect of ethylene on wheat: No. 2, untreated; No. 4, treated after 19 days.

make more frequent baking tests, so these data are admittedly scanty, but the difference between the treated and untreated wheat was so striking at first as to leave no doubt of the general trend of the results.

TABLE 1

EFFECT OF ETHYLENE TREATMENT ON VOLUME OF LOAF

When this experiment was begun the wheat had been harvested seven days and had been kept near 0°C. in the meantime.

Date	Time since harvest	Loaf volume (cold)		Remarks	
		Treated	Control	Treated	Control
	Days	cc.	cc.		
8- 3-39	7	—	2025	—	Green, coarse texture.
8- 8-39	12	2140	2070	Light in color, fine grain & texture.	Green, coarse texture.
8-15-39	19	2335	2200	Better grain & texture, color same.	Some improvement over original.
9-28-39	60	—	2225	—	Normal loaf of bread.

The influence of ethylene in improving the quality of the bread is evidently exerted on the grain, and not on the flour, as evidenced by the observation that the flour from untreated wheat gave not only a poor bread, as stated, but continued to do so after the milled flour (patent) had been exposed to 1/1,000 ethylene, in the same way as the wheat was treated. Such flour does improve somewhat with age, as does flour from the untreated wheat, but no particular differences in

behavior were noticeable. Bailey and Fifield (1939) obtained similar results on the treatment of flour with ethylene.

Experiments which it was thought might show an increased rate of metabolism in the treated wheat failed to indicate this. Thermos jugs holding about 2.5 pounds of wheat were filled with treated and untreated specimens and the rise in temperature of each was observed over a twenty day period. No particular difference was noted, however. Furthermore, oxygen-absorption measurements made in a Warburg apparatus showed no difference between the rates of oxygen consumption by one gram of treated and one gram of untreated wheat. Experiments with whole grains are subject, however, to so many errors that in the absence of very great differences between experiment and control nothing definite can be said.

On the other hand, a marked change in the viability of the wheat, as evidenced by the usual germination test, was caused by the ethylene treatment. Green wheat, as obtained from the combine harvester, is known to give low germination values (Atkinson and Jahnke, 1918; Swanson, 1938). When treated with ethylene in the proportion of one part per thousand of air on three successive days as described before, the wheat appeared to be stimulated slightly for a few days after treatment, but thereafter lost viability on continued storage instead of gaining it, as did the control. After a single treatment with a 1/10,000 ethylene-air mixture, however, an increase in viability was observed. Unfortunately the wheat was by this time past the stage where there was much difference in bread-making quality between the treated and untreated portions. Nevertheless, the effect of ethylene on the germination was marked. The wheat behaved as though capacity for germination was increased by exposure to ethylene, but it appeared that too much ethylene was decidedly harmful in this connection. The germination tests run at 20° on 100 grains are shown in Table II as the percentage of sprouted grains in six days.

TABLE II  
GERMINATION TESTS—PERCENT GERMINATION IN SIX DAYS AT 20°

Treatment of wheat stored at room temperature	Days stored after ethylene treatment						
	0	1	2	3	4	8	23
None	50	54	48	54	56	—	—
Stored 3 days in ethylene, 1/1,000	54	52	86	68	64	—	76
Stored 3 days in ethylene, 1/10,000	62	58	74	74	—	74	—

By the time these experiments had been completed, the wheat kept in cold storage no longer behaved differently from normal wheat or that kept continuously at room temperature, so no further experiments



appear possible until a new crop is available. Whether such a process would be of practical value in handling wheat is not in the province of this laboratory to say. In any case experiments on a far greater scale would be required to demonstrate its utility. The present publication is made only as a suggestion and a matter of record.

### Summary

A necessarily brief experiment on freshly harvested combine wheat has shown that small doses of ethylene are able to mature such wheat very quickly, in the sense that it becomes suitable for bread making. No reliable data pointing to an accelerated metabolism were obtained, although it seems probable that it occurs. An immediate increase in the viability of the treated wheat was observed with a low concentration of ethylene, but there is evidence that more ethylene is decidedly harmful.

The implications of these observations may be of sufficient importance to warrant attention by chemists better equipped to study the question. If the ethylene treatment will serve as a means of hastening the well known after-ripening process popularly designated as "sweating" its possibilities of use in industrial processing are worthy of consideration.

### Acknowledgment

We are very greatly indebted to Dr. W. F. Geddes and Mr. A. J. Schwantes of the University of Minnesota Department of Agriculture, St. Paul, and Mr. R. A. Hodgson of the Southeast Minnesota Experiment Station, Waseca, Minnesota, for obtaining this wheat and attending to the shipment. We are also indebted to Mr. L. H. Bailey of the Cereal Laboratory, Food Research Division of this Bureau, for the milling and baking, and also to Mr. B. E. Rothgeb of the Agricultural Marketing Service, U. S. D. A., for milling several samples of the wheat.

### Literature Cited

- Atkinson, A., and Jahnke, S. W.  
1918 Germination of grain at various periods after threshing. *Montana Agr. Exp. Sta. Bul.* 125, p. 17.
- Bailey, C. H., and Fifield, C. C.  
1939 Private communication from C. C. Fifield.
- Chace, E. M., and Church, C. G.  
1927 Effect of ethylene on the composition and color of fruits. *J. Ind. Eng. Chem.* 19: 1135-1139.
- Chace, E. M., and Sorber, D. G.  
1928 Use of ethylene for softening Bartlett pears intended for canning and drying. *The Canner* 67: 15-16.  
1936 Treating fruits and nuts in atmospheres containing ethylene. *Food Industries* 8: 130-132.
- Denny, F. E.  
1924 Effect of ethylene upon the respiration of lemons. *Botan. Gaz.* 77: 322-329.
- Gane, R.  
1934 Production of ethylene by some ripening fruits. *Nature* 134: 1008.
- Swanson, C. O.  
1935 The story of a grain of wheat from ripening to bin. *Production Annual, Northwestern Miller* 184: 12.  
1938 Wheat and flour quality (p. 120). Burgess Publishing Co., Minneapolis.

## **BARLEY AND MALT STUDIES. VI. EXPERIMENTAL MALTING OF BARLEYS GROWN IN 1938<sup>1</sup>**

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(Read at the Annual Meeting, May 1939)

The regional investigations on the malting quality of the five standard barley varieties grown in 1938 are presented as a progress report. The study of the five varieties, Oderbrucker, Wisconsin Barbless, Velvet, Manchuria, and Trebi, has been continued in co-operation with the various states for five seasons (1934 to 1938) and has been terminated by action of the Barley Improvement Council with the study of the 1938 crop. The detailed data on the physical and chemical factors of the barleys and malts for the five standard varieties and the other barley varieties grown at the various stations during the five-year period will be presented in a later publication. However, a summary of the varietal and yearly data, and a brief discussion of the data are presented in this paper.

### **Growing and Harvesting Conditions During the Season of 1938**

Growing conditions were relatively favorable for barley development during the 1938 growing season. Soil moisture was sufficiently abundant at most of the stations to give comparatively normal growth. Continued rainfall into the ripening and harvest period, however, damaged the quality of the barley at some of the stations. The damage caused by lodging of the grain, blight disease, and high moisture content of the grain while in the shock was more severe at the stations in the central section of the spring barley area than at the western stations of this area.

In many respects, the 1938 season was similar to that of 1935. In the 1935 season, however, there was a moisture shortage during the latter part of the growing season which materially reduced the quality of the barleys grown at Lincoln, Nebr., Brookings, S. D., and Fargo, N. D. In contrast, in 1938 the moisture conditions were very favorable for barley production at these western stations, which resulted in a more uniform quality of barley at all stations than in any of the previous seasons included in the investigations.

<sup>1</sup> Based on cooperative investigations between the Division of Cereal Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture, and the Wisconsin Agricultural Experiment Station. The cooperative investigations include the agricultural experiment stations of California, Colorado, Illinois, Iowa, Michigan, Minnesota, Montana, Nebraska, North Dakota, Ohio, South Dakota, and Wisconsin, where the uniform barley varietal series have been grown each year. The Federal WPA has contributed to the research program during the past year through a grant under the University of Wisconsin WPA Natural Science Project. The United States Maltsters Association has cooperated through an industrial fellowship grant to the University of Wisconsin.

### Comparison of the Barleys and Malts from the Thirteen Stations

Thirteen locations provided barley of the five standard varieties grown in 1938. The barleys were malted, including malting controls, under approximately the same conditions as in previous years. The barleys were steeped to a moisture content of 46% and malted at 46% to 44% moisture. The average steep moisture for all barleys was 45.8% and the average moisture content of the green malts at the end of the germination period was 44.6%. The barleys and malts were analyzed for the physical and chemical factors as in the previous years. The detailed data for the physical and chemical factors of the barleys and malts are given in Table I. The discussion will be limited to some of the more important characteristics of the 1938 season's barleys and malts.

The grain yields in bushels per acre varied widely for the different stations. At the stations in the central area barley yielded about the same as the average for the five-year period. Yields at Brookings, S.D., and Lincoln, Nebr., were higher than in the previous years at these stations. The yields reported for Davis, Calif., are low for all varieties except Trebi because of shattering of kernels before the barley was mature. Likewise, Velvet was low at Fort Lewis, Colo., as a result of shattering and insect injury.

The barleys from all of the stations were relatively plump and mature. The barleys from Lincoln, Nebr., Brookings, S. D., and Fargo, N. D., were superior in quality to those of any of the previous seasons except perhaps the barleys grown at these stations in 1934. The barleys grown at Waseca, Minn., and Madison, Wis., for the most part were not equal in quality to those grown at these locations in 1935. The barleys from East Lansing, Mich., and Urbana, Ill., were similar in quality to those of the three previous seasons. The varieties produced at DeKalb, Ill., were more like those grown in 1936, in quality. And, finally, the barleys from Bozeman, Mont., Fort Collins and Fort Lewis, Colo., and Davis, Calif., were, in general, similar in quality to those of the previous season's crops.

The bushel weight of the barleys varied considerably at the different stations. The bushel weights of all the barleys grown at Madison, Wis., and of Velvet and Trebi grown at East Lansing, Mich., were abnormally low, largely because of poor threshing and partly germinated kernels, rather than a large amount of thin barley. These conditions were responsible for the similarity of bushel weight of barley and malt in the lots mentioned above, as the cleaning of the malt removed the awns and rootlets and loose hulls that were instrumental in lowering the bushel weight of barley.

TABLE I  
THE PHYSICAL AND CHEMICAL FACTORS OF BARLEYS AND MALTS FOR THE FIVE STANDARD VARIETIES  
GROWN AT THE VARIOUS STATIONS IN 1938

Location and variety	Yield of barley per acre	Bushel weight of barley, dry basis	Kernel weight of barley, dry basis	Total protein in barley, $\times 6.25$	Ash content of barley	Hull content of barley	Moisture content of steeped barley	Moisture content of green malt	Recovery of malt from barley, dry basis	Bushel weight of malt, 4°C dry basis	Kernel weight of malt, dry basis	Moisture content of malt	Extract fine grind, dry basis	Time conversion	Dia-static power	Total nitrogen in malt $\times 6.25$	Total protein in malt, $\times 6.25$	Soluble nitrogen in wort	Soluble nitrogen in malt to nitrogen	Formol nitrogen relation to malt nitrogen
	Bu.	Lbs.	Mg.	%	%	%	%	%	%	Lbs.	Mg.	%	%	Min.	°L.	%	%	%	%	%
<b>Oderbrucker, Ped. 5-1</b>																				
East Lansing, Mich.	—	38.6	29.9	13.06	2.91	13.6	45.1	46.5	85.7	33.9	26.3	5.1	73.6	5	133	2.13	13.31	.708	33.2	6.3
Urbana, Ill.	33.7	39.3	29.3	11.58	3.05	15.0	46.5	44.6	87.3	35.7	26.6	5.0	75.5	7	154	1.84	11.52	.731	39.7	8.0
DeKalb, Ill.	30.5	38.5	27.7	11.70	3.26	12.6	46.3	44.5	87.4	36.2	24.4	5.2	76.1	7	168	1.81	11.32	.779	43.0	8.5
Madison, Wis.	17.7	33.6	24.8	14.61	3.36	13.9	46.5	43.4	90.0	33.9	22.6	4.9	72.7	<5	238	2.42	15.12	.895	37.0	7.4
Waseca, Minn.	43.7	38.6	25.4	13.72	3.39	12.5	47.0	45.2	85.8	34.7	23.0	5.7	73.1	7	148	2.12	13.25	.771	36.4	6.9
Kanawha, Iowa	24.8	37.8	23.5	13.67	3.82	16.3	47.6	45.3	86.5	34.4	21.1	4.9	73.4	<5	208	2.32	14.50	.906	39.1	8.4
Fargo, N. D.	30.3	39.0	23.2	13.94	3.50	14.5	45.7	48.7	84.6	32.3	20.2	5.8	72.0	<5	240	2.63	16.44	.897	34.1	8.5
Brookings, S. D.	30.4	40.0	25.6	17.80	3.02	13.6	45.9	45.0	80.7	35.0	21.9	5.2	72.3	<5	270	2.97	18.56	.980	32.9	8.3
Lincoln, Neb.	21.5	41.3	25.1	16.36	2.91	12.9	45.7	45.5	86.4	33.9	21.9	5.2	71.5	5-7	239	2.62	16.38	.861	32.9	8.6
Bozeman, Mont.	58.8	47.0	36.3	15.25	2.84	10.7	44.8	45.0	86.5	39.3	31.7	5.8	75.3	<5	256	2.52	15.75	.891	35.4	7.1
Fort Collins, Colo.	49.6	46.5	32.5	13.97	2.81	10.6	44.6	44.9	85.8	38.5	27.8	6.0	76.5	<5	224	2.39	14.94	.799	33.4	6.4
Fort Lewis, Colo.	50.9	41.3	32.1	12.78	3.19	11.0	45.6	44.3	85.7	40.8	28.6	5.8	76.4	5	182	2.09	13.06	.701	33.5	6.7
Davis, Calif.	12.5*	44.1	32.0	14.63	2.94	11.7	44.0	41.1	89.2	40.3	29.8	5.5	73.2	5	170	2.44	15.25	.687	28.2	5.6
Average		40.4	28.3	14.21	3.15	13.0	45.8	44.9	86.7	36.4	25.1	5.4	74.0	5.1	224	2.33	14.57	.816	35.2	7.0
<b>Wisconsin Barless Ped. 38</b>																				
East Lansing, Mich.	—	38.8	29.6	12.22	2.79	14.5	46.4	45.2	87.1	36.4	27.2	5.5	72.8	7-10	137	2.10	13.13	.542	25.8	4.8
Urbana, Ill.	27.4	38.2	31.3	11.90	3.09	15.6	46.3	44.3	87.9	36.0	26.1	5.2	73.1	7	129	1.91	11.96	.619	32.4	6.0
DeKalb, Ill.	34.8	38.2	26.5	11.46	3.12	14.4	45.9	44.1	87.5	35.9	24.3	5.1	72.4	7	119	1.74	10.88	.661	38.0	6.9
Madison, Wis.	32.9	35.0	28.1	14.36	3.18	14.8	45.2	42.8	89.3	35.0	23.7	5.2	70.4	7	151	2.39	14.94	.671	28.1	7.0
Waseca, Minn.	36.8	38.6	25.7	12.95	3.24	14.1	46.9	45.0	87.2	35.7	23.7	5.8	72.8	7	154	2.18	13.63	.648	29.7	4.9
Kanawha, Ia.	39.9	39.4	24.9	12.95	3.70	16.2	45.9	45.5	86.8	35.5	22.7	5.7	72.7	7	144	2.15	13.44	.679	31.6	5.4
Fargo, N. D.	46.5	40.4	27.2	14.78	3.29	13.5	45.7	45.1	87.2	35.8	23.9	5.6	72.9	5	147	2.44	15.25	.610	25.0	4.4
Brookings, S. D.	49.5	41.2	28.6	15.38	2.92	12.3	44.4	41.6	85.2	37.0	24.9	5.6	72.9	5	160	2.54	15.86	.663	26.1	5.1
Lincoln, Neb.	38.8	41.2	25.0	14.43	2.86	16.0	45.2	45.4	85.0	35.8	22.5	5.9	71.6	7	161	2.38	14.88	.679	28.5	4.9
Bozeman, Mont.	70.0	46.7	38.7	14.66	2.80	12.5	44.4	44.6	86.9	40.6	34.8	6.3	73.9	5	181	2.38	14.88	.587	24.7	4.5
Fort Collins, Colo.	67.7	45.4	32.0	15.25	2.80	11.5	44.4	44.8	87.2	39.3	27.7	6.1	73.4	5	158	2.45	15.31	.646	26.4	4.9
Fort Lewis, Colo.	77.7	43.5	33.1	11.88	3.02	11.9	45.6	44.4	89.2	38.9	30.3	5.7	74.8	10	111	1.77	11.08	.491	27.7	4.9
Davis, Calif.	17.8*	43.5	33.7	13.06	2.84	12.2	45.9	43.7	87.0	41.0	29.2	5.8	75.2	10	127	2.19	13.69	.577	26.3	4.3
Average		40.8	29.6	13.48	3.05	13.8	45.6	44.6	87.2	37.1	26.1	5.6	73.0	7.1	145	2.20	13.76	.621	28.5	5.2

\* Low yields due to the kernels dropping from heads before harvest.

TABLE I—Continued

Location and variety	Yield of barley per acre	Bushel weight of barley, dry basis	Kernel weight of barley, dry basis	Total protein in barley, $\times 6.25$	Ash content of barley	Hull content of barley	Moisture content of steeped barley	Moisture content of green malt	Recovery of malt from dry basis	Bushel weight of malt, 4% basis	Kernel weight of malt, dry basis	Moisture content of malt	Extract of fine grind, dry basis	Time conversion	Dia-static power	Total nitrogen in malt	Total protein in malt, $\times 6.25$	Soluble nitrogen in wort	Soluble nitrogen in relation to malt nitrogen	Formol nitrogen in relation to malt nitrogen
	Bu.	Lbs.	Mg.	%	%	%	%	%	%	Lbs.	Mg.	%	%	Min.	°L.	%	%	%	%	%
<b>Velvet, Minn. 447</b>																				
East Lansing, Mich.	—	35.7	29.7	12.44	3.05	14.8	45.6	45.4	85.8	34.8	25.9	5.3	74.4	5	146	2.04	12.75	717	35.1	6.6
Urbana, Ill.	27.0	38.9	28.4	12.13	3.05	14.9	45.9	43.9	88.0	35.6	24.5	5.1	74.8	5	150	1.91	11.86	770	40.3	8.0
DeKalb, Ill.	29.7	38.9	26.2	12.02	3.30	13.2	46.3	44.1	88.4	36.0	24.0	5.1	76.2	5	136	1.90	11.90	792	41.7	8.0
Madison, Wis.	23.8	34.4	23.1	14.73	3.13	13.4	47.3	41.8	91.1	34.5	21.2	5.0	71.6	<5	183	2.47	15.44	885	35.8	7.0
Waseca, Minn.	47.7	39.0	23.5	13.32	3.64	13.2	46.7	44.6	87.8	36.0	20.2	5.6	73.1	5	200	2.24	14.00	842	37.6	8.0
Kanawha, Ia.	39.8	39.1	23.3	13.38	3.70	14.4	47.0	45.1	88.3	34.0	20.7	5.1	72.3	5	205	2.17	13.56	846	39.0	8.4
Fargo, N. D.	38.9	42.7	25.4	15.16	3.29	13.2	43.6	45.2	86.2	36.5	21.7	5.2	72.0	<5	208	2.52	15.75	841	33.4	7.0
Lincoln, Neb.	24.8	40.1	23.7	15.91	3.14	16.5	45.4	44.7	85.5	36.1	21.1	5.3	74.4	5	215	2.72	17.06	898	33.0	6.7
Roseman, Mont.	73.2	47.5	35.4	15.56	2.94	16.7	46.3	45.8	84.8	36.3	21.3	5.7	72.8	<5	221	2.60	16.25	893	34.3	6.4
Fort Collins, Colo.	59.4	44.0	31.6	16.00	2.96	11.4	44.0	44.8	86.7	39.7	31.3	6.2	75.7	<5	231	2.50	15.63	799	32.0	6.2
Fort Lewis, Colo.	36.4*	40.5	32.2	12.08	3.06	12.6	45.6	44.6	87.1	38.6	28.2	5.7	73.8	<5	243	2.37	16.06	866	33.7	6.6
Davis, Calif.	13.9*	43.6	31.7	13.38	2.98	13.3	44.0	42.3	89.3	39.3	29.2	5.0	73.7	5	164	2.20	13.72	677	30.8	6.2
<b>Average</b>		40.5	27.6	13.95	3.17	14.1	45.6	44.4	87.4	36.5	24.4	5.4	74.0	5.0	188	2.29	14.32	811	35.7	7.1
<b>Manchuria, N. D. 2121</b>																				
East Lansing, Mich.	—	38.5	29.3	13.72	3.00	11.8	48.5	45.9	87.1	35.7	26.3	5.6	74.1	<5	212	2.30	14.36	823	35.8	7.5
Urbana, Ill.	25.0	37.6	27.3	13.19	3.11	11.7	45.6	44.7	87.7	34.8	24.0	4.9	74.3	<5	169	2.10	13.13	852	40.6	8.1
DeKalb, Ill.	30.0	37.4	26.4	12.02	3.36	12.8	46.3	44.1	87.9	34.7	23.1	5.0	75.1	<5	158	1.98	12.40	856	43.2	9.3
Madison, Wis.	19.0	32.6	23.4	15.25	3.17	14.2	47.3	43.6	91.0	32.3	20.9	5.0	73.7	<5	257	2.59	16.19	991	38.3	8.0
Waseca, Minn.	41.5	36.9	23.2	13.86	3.56	15.9	47.7	45.3	86.7	33.5	20.9	5.0	73.5	<5	209	2.31	14.43	975	42.2	9.4
Kanawha, Ia.	26.1	23.0	13.86	3.69	3.69	13.9	47.6	45.5	86.7	34.6	20.6	5.2	73.0	5	223	2.24	13.99	882	39.4	8.3
Fargo, N. D.	46.4	42.3	25.4	13.94	3.06	12.0	45.1	44.7	87.1	36.1	22.8	5.1	74.3	7	205	2.31	14.44	740	32.0	6.1
Brookings, S. D.	28.2	39.5	23.7	16.34	2.91	11.9	45.2	44.3	86.9	35.0	20.9	5.3	73.4	5	241	2.76	17.24	915	33.1	6.8
Lincoln, Neb.	24.0	40.9	22.6	15.75	2.95	12.9	45.7	45.5	86.6	35.2	20.8	5.0	72.2	<5	248	2.54	15.88	906	34.7	7.3
Roseman, Mont.	66.1	46.5	35.6	14.97	2.86	9.7	44.4	45.6	85.8	38.3	30.1	6.3	75.9	<5	244	2.49	15.56	866	35.8	6.9
Fort Collins, Colo.	54.2	44.9	29.4	14.59	2.81	13.5	45.4	45.0	87.1	37.4	25.8	5.4	76.4	<5	238	2.46	15.38	866	35.2	6.9
Fort Lewis, Colo.	53.6	41.6	31.1	13.06	3.16	13.8	45.6	45.4	87.2	37.9	27.4	5.4	76.4	5	183	2.12	13.25	773	36.5	7.1
Davis, Calif.	18.0*	44.8	32.0	13.56	2.80	10.3	44.0	41.7	90.6	40.9	30.1	5.7	75.0	5	164	2.20	13.72	654	29.7	5.8
<b>Average</b>		40.1	27.1	14.16	3.11	12.6	46.0	44.7	87.6	35.9	24.1	5.3	74.3	4.5	212	2.36	14.73	854	36.4	7.5

TABLE I—Continued

Location and variety	Yield of barley per acre	Bushel weight of barley, dry basis	Kernel weight of barley, dry basis	Total protein in barley, N $\times 6.25$	Ash content of barley	Hull content of barley	Moisture content of steeped barley	Moisture content of green malt	Recovery of malt from barley, dry basis	Bushel weight of malt, 4% basis	Kernel weight of malt, dry basis	Moisture content malt	Extract fine grind, dry basis	Time conversion	Dia-static power	Total nitrogen in malt	Total protein in malt, N $\times 6.25$	Soluble nitrogen in wort	Soluble nitrogen in malt nitrogen	Formol nitrogen to malt nitrogen
	Bu.	Lbs.	Mg.	%	%	%	%	%	%	Lbs.	Mg.	%	%	Min	°L.	%	%	%	%	%
Trebi	—	35.8	36.6	11.75	3.04	13.7	46.1	44.0	88.9	35.8	33.9	5.6	74.6	10	150	1.96	12.25	.596	30.4	5.8
East Lansing, Mich.	—	35.8	36.6	11.75	3.04	13.7	46.1	44.0	88.9	35.8	33.9	5.6	74.6	10	150	1.96	12.25	.596	30.4	5.8
Urbana, Ill.	33.0	38.9	37.7	13.56	3.12	13.2	46.7	44.3	87.8	35.5	29.0	5.2	73.1	7	124	2.11	13.19	.745	35.3	7.0
DeKalb, Ill.	40.3	30.4	37.7	9.33	3.10	13.2	45.7	44.2	88.4	36.9	33.5	5.4	78.8	10	108	1.50	9.38	.620	41.3	8.6
Madison, Wis.	27.9	31.9	31.2	14.25	2.87	14.9	47.7	42.7	91.6	33.5	27.6	5.2	70.8	5	178	2.37	14.81	.738	31.1	5.8
Waseca, Minn.	50.3	35.7	30.5	11.69	3.47	17.6	47.7	44.8	87.7	34.1	28.7	5.7	73.8	7	167	2.02	12.63	.675	33.4	6.4
Kanawha, Ia.	34.6	37.7	30.4	12.51	3.58	16.9	46.8	44.9	88.0	34.2	28.1	5.7	73.8	7	154	1.94	12.13	.677	34.9	6.2
Fargo, N. D.	53.5	41.7	33.8	15.06	2.93	13.6	45.6	44.2	89.7	36.8	30.4	5.4	72.9	10	194	2.44	15.25	.618	25.3	4.3
Brookings, S. D.	41.2	40.3	32.8	14.59	2.54	14.7	45.9	44.1	88.9	33.9	29.1	5.6	69.6	5	177	2.44	16.50	.697	28.5	5.5
Lincoln, Neb.	41.6	38.7	28.2	15.61	2.91	17.9	45.4	45.2	87.0	36.0	24.6	5.4	69.6	5	217	2.64	16.50	.697	26.4	4.7
Bozeman, Mont.	100.8	46.3	45.4	14.03	2.85	10.2	44.0	43.9	89.5	40.2	40.8	6.2	75.7	7	211	2.30	14.38	.564	24.5	4.1
Fort Collins, Colo.	87.4	44.6	39.6	11.66	2.51	13.9	44.8	43.5	89.8	38.4	36.3	6.1	78.0	7-10	161	1.95	12.19	.587	30.1	4.9
Fort Lewis, Colo.	85.2	44.0	43.3	10.66	2.95	11.7	45.6	44.6	89.6	40.3	39.1	6.2	78.1	20	144	1.78	11.13	.461	25.9	4.1
Davis, Calif.	60.6	41.0	37.2	12.56	2.80	14.6	45.6	43.7	87.0	38.8	33.2	5.8	74.2	20	124	2.17	13.56	.527	24.3	4.4
Average		39.7	35.3	12.87	2.97	14.3	46.0	44.2	86.8	36.5	31.7	5.7	74.3	9.3	162	2.12	13.28	.631	30.1	5.5
Grand Average		40.3	29.6	13.73	3.09	13.6	45.8	44.6	87.5	36.5	26.3	5.5	73.9	6.1	188	2.26	14.13	.746	33.2	6.5

The kernel weight of the barleys was relatively high for all five varieties. The varietal ranking for the average kernel weight of barley from all stations was the same as in the previous years. The four varieties, Wisconsin Barbless, Oderbrucker, Velvet, and Manchuria, showed some shifting in rank at the individual stations.

The protein content of the barleys varied widely between the central and western stations of the spring barley area. The protein content of the barleys grown at Madison was relatively high. The barleys from Fargo, Brookings, Lincoln, and Bozeman were all high in protein content in contrast to those from the other stations. Protein content varied from 9.3% for Trebi grown at DeKalb to 17.5% for Oderbrucker grown at Brookings.

Ash content of the barleys was relatively high and fairly uniform at all stations. The varieties were very similar in ash content; the maximum variation in average ash content for the five varieties was 0.2%.

Hull content of the barleys showed considerable variation at the different stations. In general, the average hull content at the stations in the central area was slightly higher than the five-year average, whereas the barleys from Brookings, Lincoln, and Fargo were below the average at these stations.

Recovery of malt from barley showed a difference in varietal trend from the previous years. The average recovery from the Wisconsin Barbless variety in 1938 was not greatly different from that of Oderbrucker, Velvet, and Manchuria, whereas, in the previous years recovery was higher for this variety than for the other three. Trebi averaged highest in recovery as in the previous years except 1935, when Wisconsin Barbless exceeded it.

The extract content of the malts was comparatively high except for the barleys grown at Madison and Lincoln. The varietal ranking for the average extract content was the same as in the previous years. There was some shifting in the varietal ranking at the individual stations; however, Wisconsin Barbless malts were the lowest in extract content, while the Manchuria and Trebi malts were highest.

The diastatic power of the malts was relatively high for all of the varieties and at most of the stations. The malt lowest in diastatic power, 108° L., was from Trebi from DeKalb. The highest, 270° L., was from Oderbrucker grown at Brookings. These two barleys were likewise the lowest and highest, respectively, in protein content. The varieties ranked the same for the average diastatic power as in the previous years. There was also less shifting in rank between the varieties at the individual stations than for many other factors.

The conversion time on the malts was relatively rapid. The average conversion time was similar to that of 1935.

The soluble nitrogen in the wort as well as the soluble nitrogen as percentage of malt nitrogen was relatively high for the malts from most of the stations. The varietal ranking for average soluble nitrogen in the wort was similar to the previous year. There was as usual a marked difference between varieties in the soluble nitrogen in the wort as percentage of malt nitrogen. Permanently soluble nitrogen and formol nitrogen expressed as the percentage of the malt nitrogen showed variations similar to soluble nitrogen in the wort for both varieties and stations.

The malts from the five standard varieties grown at the 13 locations showed less variation in quality between stations than in any of the previous years studied. The varieties have maintained essentially the same ranking based on the average for all stations as in the previous years for the major characteristics studied. There was, however, greater fluctuation in the rank of the varieties at the individual stations than in the previous year.

#### **Five-Year Comparison of the Barleys and Malts from Six Stations**

The comparative averages for the barleys and malts from the six stations which have supplied barley of the five standard varieties for each of the five years show the reaction of the varieties for the five-year period and the influence of seasonal conditions on these barleys. The six stations from which the data are included in the average are: (1) East Lansing, Mich., (2) Madison, Wis., (3) Waseca, Minn., (4) Kanawha, Iowa, four years, Emmetsburg, Iowa, one year, (5) Brookings, S. D., and (6) Bozeman, Mont. The varietal averages for the five years (1934-38) and the averages for the five varieties combined for each year are given in Table II. The analysis of variance of some physical and chemical factors of barleys and malts for the same varieties and stations for the five-year period are given in Table III.

The average bushel weights of barley were very similar for the four varieties, while that for Trebi was lower. The bushel weights varied considerably between the individual stations. The variation between stations and between years was much greater than the variation between varieties. However, the varieties held the same position at each of the locations.

The average kernel weight of barley showed a varietal difference for the five-year average at the six stations. The varieties had approximately the same ranking as in the average for the larger number of stations for each year. The yearly averages of all five varieties showed a higher kernel weight of barley at these six stations in 1934,



TABLE II

AVERAGES FOR THE FIVE STANDARD VARIETIES FOR THE FIVE YEARS 1934 TO 1938 AND YEARLY AVERAGES FOR THE FIVE VARIETIES COMBINED FOR THE BARLEYS GROWN AT THE SIX STATIONS: EAST LANSING, MICH., MADISON, WIS., WASECA, MINN., KANAWHA AND EMMETSBURG, IOWA, BROOKINGS, S. D., AND BOZEMAN, MONT.

	Varietal average for five years (1934-38)					Yearly averages for five varieties				
	Oderbrucker	Wisconsin Barbless	Velvet	Manchuria	Trebi	1934	1935	1936	1937	1938
Bushel weight barley, dry basis, lbs.	39.6	39.7	39.9	39.3	38.7	39.4	41.1	40.0	37.8	39.0
Kernel weight Barley, dry basis, mg.	26.1	27.7	25.5	24.9	33.7	29.8	27.0	26.3	26.1	28.7
Hull content barley, dry basis, %	13.8	14.0	14.2	12.8	13.9	12.5	10.9	13.3	15.8	13.7
Recovery malt from barley dry basis, %	87.0	88.6	88.1	87.1	89.5	87.8	86.4	89.2	89.2	87.6
Bushel weight malt, 4% basis, lbs.	35.9	36.7	36.6	35.7	35.7	36.1	37.4	35.3	34.2	35.7
Kernel weight malt, dry basis, mg.	23.1	26.8	22.6	22.0	30.6	27.1	23.5	23.5	23.5	25.7
Extract content, dry basis, %	72.3	70.5	71.8	72.8	72.5	71.6	72.7	70.8	71.2	73.4
Total protein in malt, %	15.5	14.5	14.8	15.3	13.9	16.5	14.3	14.3	14.2	14.6
Soluble nitrogen in wort as protein, %	5.4	4.0	5.0	5.5	4.1	5.0	5.0	4.6	4.6	4.9
Soluble nitrogen in relation to malt nitrogen, %	34.7	27.5	34.6	35.9	29.4	29.7	35.0	31.6	32.6	33.2
Conversion time, min.	5.4	8.3	5.6	5.5	10.0	7.7	5.9	6.2	9.6	5.4
Diastatic power, °L	192	124	153	191	145	109	164	168	168	195

TABLE III

ANALYSIS OF VARIANCE (F VALUES) OF PHYSICAL AND CHEMICAL FACTORS OF BARLEYS AND MALTS FOR THE FIVE STANDARD <sup>1</sup> VARIETIES GROWN AT SIX STATIONS <sup>2</sup> FOR THE FIVE YEARS 1934 TO 1938

Source	Deg. freedom	Bushel weight of barley	Kernel weight of barley	Estimated time to reach 46% moisture in steep	Recovery of malt from barley	Extract in malt, dry basis	Protein in malt	Soluble nitrogen in wort	Soluble nitrogen as per cent of malt nitrogen	Conversion time in mashing	Diastatic power of malt	5% point	1% point
Varieties	4	5.4	226.9	13.1	12.9	12.4	22.7	128.5	59.1	20.6	70.0	2.49	3.56
Years	4	337.4	46.0	51.3	16.6	17.5	65.7	53.7	23.3	3.2	78.2	2.49	3.56
Stations	5	37.4	261.0	8.3	3.7	36.1	52.9	8.9	13.4	13.4	27.5	2.33	3.26
Var. × Sta.	20	1.3	0.7	1.2	0.5	0.5	1.7	1.7	0.4	1.1	1.6	1.73	2.16
Var. × Year	16	2.6	1.28	1.2	1.0	1.0	2.9	1.8	0.7	1.4	2.4	1.81	2.29
Sta. × Year	20	31.2	29.2	13.2	7.2	11.5	24.6	12.3	2.6	4.3	9.8	1.73	2.16
Error	80	—	—	—	—	—	—	—	—	—	—	—	—
Total	149	—	—	—	—	—	—	—	—	—	—	—	—

<sup>1</sup> Varieties: Oderbrucker, Wisconsin Barbless, Velvet, Manchuria, and Trebi.

<sup>2</sup> Stations: East Lansing, Mich., Madison, Wis., Waseca, Minn., Kanawha and Emmetsburg, Iowa, Brookings, S. D., and Bozeman, Mont.

1935, and 1938 than in the dry season of 1936, and in 1937 when stem rust was severe at four of the stations.

The average protein content of the barleys and malts showed varietal, seasonal, and station differences. Trebi ranked lowest in protein content, while Wisconsin Barbless, Velvet, Manchuria, and Oderbrucker following in ascending order. The last three varieties were relatively similar and occasionally changed rank in the yearly and station averages. The average protein content for 1935-38 at the six stations was relatively similar, while the 1934 average was appreciably higher.

The average hull contents of the five varieties were similar except for Manchuria which was lower than the other varieties. The average for the five years varied from 10.9 in 1935 to 15.8% in 1937, the season that stem rust was severe on barley through the spring barley area. The average hull content for 1938 was somewhat higher than for the first three years.

The average recovery of malt from barley showed a varietal and seasonal difference. The varietal ranking for recovery arranged in descending order was as follows: Trebi, Wisconsin Barbless, Velvet, Manchuria, and Oderbrucker; the latter two varieties were not significantly different in recovery. Average recovery was lowest in 1935, and highest in 1936 and 1937.

Average bushel weight and kernel weight of malt showed a fair agreement with bushel weight and kernel weight of barley in both the varietal and seasonal averages. The seasonal averages for bushel weight of malt showed greater variations than in the averages for the varieties. The average kernel weight of malts from the six stations indicates three varietal groups: Trebi 30.6 mg., Wisconsin Barbless 26.8 mg., and Oderbrucker, Velvet, Manchuria 23.1, 22.6, 22.0 mg. respectively. The average kernel weights of malt in 1934 and in 1938 were significantly higher than in the other three years.

The average extract content showed varietal, station, and seasonal differences at the six stations. The varietal ranking in ascending order was as follows: Wisconsin Barbless, Velvet, Oderbrucker, Trebi, and Manchuria. The varietal ranking of the last three varieties varied slightly over the larger number of stations and in individual years, but this variation was not significant. The average extract content for the five varieties combined for each of the five years indicated that the seasons of higher moisture, namely 1935 and 1938, were the more favorable for the production of high extract.

The average soluble nitrogen in the worts and in percentage of the total malt nitrogen showed marked varietal, station, and yearly differences. Both Wisconsin Barbless and Trebi malts were significantly lower than the malts of the other three varieties. The lower values for these two varieties have been consistent at the different stations and in all years. The other three varieties changed positions at the different stations, and in different years also showed differences, in that 1934 was low and other years were intermediate or high in soluble nitrogen as percentage of malt nitrogen.

The averages for diastatic power of the malts suggested the same general varietal, station, and seasonal responses as shown for soluble nitrogen in the wort as percentage of malt nitrogen and as shown for conversion time. The same two varieties, Wisconsin Barbless and

Trebi, were significantly lower than the other three. The average diastatic power for the season of 1938 was appreciably higher than for the previous three years (1935-37) in which the seasonal averages were very similar. The low average for 1934 was caused mostly by the method of drying the malts in which the moisture content was reduced to an average of 2.9%.

In summation, the malts from the barleys grown in 1938 at all stations were in general of uniformly better quality than in any of the previous years included in the regional study. The 1938 season perhaps represents the first year in the five when growing conditions were favorable over the entire area represented by the cooperating stations. There have been two years at least at each station when seasonal conditions have been favorable for the development of good-quality barley. There likewise have been two years at most of the stations in the area when the growing conditions have been very unfavorable for barley production. The five-year survey has shown that regardless of these wide variations in environmental conditions the varieties maintain their relative ranking, except as noted, for most of the characteristics used in evaluating quality.

## BOOK REVIEWS

**A Physiological Study of the Winter Wheat Plant at Different Stages of Its Development.** By Edwin C. Miller. Technical Bulletin No. 47 of the Kansas Agricultural Experiment Station, Manhattan, Kansas. 167 pages. 1939.

This extensive bulletin deals with studies of certain phases of the metabolism of the nitrogen, phosphorus, potassium, and carbohydrates of the winter wheat plant from "seed to seed." Whereas much of the published data on this subject is representative of limited analyses performed during one growing season, Dr. Miller presents in this bulletin the results of adequate sampling throughout four crop years. The appendix of 84 pages of tables gives some idea of the scope of this investigation. For additional clarity full use of figures is made throughout the text. Climatological data and an adequate bibliography of 92 literature citations make the bulletin a complete presentation.

Analyses were made on the grain before planting early in October, then on the growing plant at two- or three-week intervals throughout the winter. After initiation of rapid "spring growth" in April sampling was conducted at weekly intervals until the plants were mature with ripe grain in June. During the last growth period of grain development and ripening, data are presented for the chaff and grain as well as for the stems and leaves. The data include analyses for total and protein nitrogen, total and water soluble phosphorus, total potassium, reducing and nonreducing sugars, starches, and hemicelluloses. All analyses are expressed both on the basis of percentage of dry weight and as grams per 100 plants. Some might wish that certain constituents, for instance the water-soluble carbohydrates, were expressed on the basis of green weight or as percentage in the water present in the growing plant. However the adequate data permit recalculation by those particularly interested in this phase.

Dr. Miller's very comprehensive work will be of great value not only to the physiologist interested in the metabolism of the winter wheat plant throughout the early periods of its development but likewise to the cereal chemist whose interest is perhaps more strongly directed to the elaboration of proteins and carbohydrates in the ripening grain.

ERIC KNEEN

**Physical Tests of Dough Quality.** By C. H. Bailey. Published as Volume XVI, No. 6, of *Wheat Studies*, by the Food Research Institute, Stanford University, California. March, 1940. 57 pages with illustrations. Price \$1.25.

This is a thoroughgoing account of what has been accomplished to date in the application of mechanical techniques for the characterization and measurement of those physical dough properties that are of major interest and importance to the cereal technologist. There are 129 literature citations.

Divided into three sections, the first deals with flour absorption in relation to properties such as viscosity, mobility, elasticity, tensile strength, extensibility and stickiness. These properties, respectively, are defined and given mathematical expression wherever the latter is possible. Most of the methods and devices that have been proposed at various times for the estimation of water absorption are described, including viscometers, plastometers, extensimeters, dough mixers, etc.

The second section discusses physical tests of crude gluten, describing specifically the several types of apparatus and procedure (starting with Boland in 1848) that have been devised and recommended for the characterization of gluten properties. The reader is cautioned that gluten, after all, is only one dough constituent, and that its properties may not always necessarily serve as a completely satisfactory index to dough characteristics.

The largest of the three sections is the one on physical tests of wheat-flour dough. This is divided into subsections dealing, respectively, with recording dough mixers, ductility measurements, extensimeters, and the extensograph. The principles underlying each method and instrument are fully explained, and the more important of these are illustrated by pictures, diagrams, and charts. The author considers it highly unlikely that any one instrument or any single test can ever be expected to serve as a basis for the complete appraisal of dough quality and baking behavior. "Rather must we expect, for some time to come, that several tests must be applied, notably through the progress of a fermentation period, to determine how a dough is responding with the lapse of time, and in the face of ensuing biochemical events."

In the preparation and publication of this monograph, both the author and the Food Research Institute have rendered a noteworthy and timely service to cereal technologists.

M. J. BLISH

**World Wheat Planning, and Economic Planning in General.** By Paul de Hevesy. Oxford University Press, London. 910 pages. Price 38/-.

Of this large volume, only the prospectus of which has been made available to the reviewer, Part I states the world wheat problem with its implications, discusses the economic factors affecting it, and proposes a world plan involving price agreement, acreage adjustment, disposal of surpluses, etc. Part II discusses the merits of the competitive system versus state planning and control of goods and services in general and in the light of specific economic conditions and trends. The future of agriculture is dealt with. Part III takes up the agricultural policies of 47 individual nations, respectively.

The book is represented to be a unique and comprehensive encyclopedia of factual data on wheat production, acreage, yield, trade consumption, stocks, transport, costs, prices, and sources of information. These data are presented in 56 appendices covering some 200 pages.

M. J. BLISH

# CEREAL CHEMISTRY

VOL. XVII

SEPTEMBER, 1940

No. 5

## THE ACTION OF OXIDIZING AND REDUCING AGENTS ON FLOUR

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(Received for publication May 2, 1940)

In previous publications from this laboratory (Sullivan, Howe, and Schmalz, 1936; Sullivan and Howe, 1937), we have shown that the presence of glutathione was responsible for the harmful action of germ on wheat flour. It would be logical to assume that low-grade flours owe their poorer baking quality, in part at least, to the presence of small amounts of germ which cannot be completely removed in milling and which contain glutathione. Indeed, by potentiometric titration, the low grade showed the presence of more reducing substances than the clear and the clear more than the patent.

It was soon apparent, however, that all flours which responded to bromate or Agene did not show the amount of reducing substance to be expected. Certain low-grade flours give a faint nitroprusside reaction (test for S-H) but the clear and especially the patent give tests that are negative or not clearly positive. Furthermore, certain mill streams such as the break flours which show an enormous response to oxidation give negative nitroprusside tests in the flour and in the dough. Titration values as shown in Table I support this. At first

TABLE I  
COMPARISON OF THE BROMATE RESPONSE IN BAKE AND THE  
AMOUNT OF BROMATE REDUCED

Sample	Protein (13.5% moisture)	Response to KBrO <sub>3</sub> in bake	0.01 N KBrO <sub>3</sub> used per 20 g. sample
	%	cc.	cc.
Patent	12.90	+ 80	0.80
Clear	16.10	+370	1.05
Low grade	17.50	+350	1.50
4th midds	12.40	- 10	0.80
3rd break	16.60	+510	0.95
2nd chunks	15.90	+480	1.80
Germ (N × 6.25)	30.00	—	50.00
Bran (N × 6.25)	17.80	—	3.80

we thought that this might be attributed to the fact that glutathione, or some other substance containing a reducing group, was combined in such a way that even its qualitative identification was made impossible. There is no question but that the harmful effect of wheat germ on flour is due to its content of glutathione, but as the work progressed on flour, the problem became much more complicated. We have not been able to show that flours with poorer baking quality always have a higher glutathione content.

Jørgensen (1935), as well as Balls and Hale, believes that flour contains "powerful but latent" proteolytic enzymes and that the proteolytic enzymes are of the papain type. Papain is activated by SH compounds, hydrogen sulfide, and hydrocyanic acid, and inactivated by certain chemicals such as bromate, iodate, and ascorbic acid. Flour is weakened by the former compounds and strengthened by the use, in proper amounts, of the latter compounds. Balls and Hale (1938) have concentrated a proteolytic enzyme from an extract of wheat bran and concluded that it is an enzyme of the papain type. They have also obtained some concentration of the proteinase from flour although the latter was difficult to purify.

Jørgensen (1938) has attempted to support his theory by experiments indicating the decrease in water-soluble nitrogen of flour extracts with increasing amounts of bromate. The amounts of oxidizing agents or ascorbic acid necessary to produce any marked change in the water-soluble nitrogen are far in excess of the amounts normally used for improvement in baking quality. Jørgensen says that this is because, in such extractions, the bromate, for example, is acting in approximately one-seventh of the concentration which exists in fermenting doughs and that yeast, which activates the proteolytic enzymes, is present in the dough and gives, when present in the extraction mixture, a greater increase of soluble nitrogen with smaller amounts of bromate.

Read and Haas (1939) found that tests made on gelatin showed that the proteinase of wheat malt flour is far less responsive to inhibition by bromate than are papain and bromelin. When bromate was used in quantities applicable to commercial bakery practice, no significant repression of wheat proteinase was apparent. Ford and Maiden (1938) are of the opinion that the action of glutathione is a direct action on the gluten proteins rather than an activation of the proteolytic enzymes.

Balls and Hale (1936) state that with regard to the action of certain reducing substances on gluten, it might be that there are two processes to consider, the action of cysteine and similar compounds on the protein and on the proteolytic enzyme. Balls and Hale in a later publication

(1938) state that since the proteinase of wheat is an enzyme similar to papain, its inactivation by oxidizing agents such as bromate, persulphate, metavanadate, and Agene still seems to explain the beneficial effect of such bread improvers.

Bungenberg de Jong (1938) offers the following possible explanation. The protein system of gluten interacts with lecithin, by which action indirectly the more hydrophobic substances such as fats and sterols can be bound to the protein. Unsaturated fatty acids influence the solvation of the protein lecithin complex. This total system is sensitive to oxidation-reduction processes. Whether this is a direct action on some active group or an indirect action on the surface film has yet to be demonstrated.

Baker and Mize (1937) have presented some very interesting results on mixing doughs in vacuum and in the presence of various gases such as oxygen, nitrogen and hydrogen. Doughs mixed in vacuum or inert gases showed no deterioration from long continued mixing. Such deterioration did occur when oxygen or oxygen yielding compounds were present. The action of bromate in a dough is affected, according to Baker and Mize, by the amount of mixing a dough has received.

According to Freilich and Frey (1937) the incorporation of oxygen into bread doughs inhibits proteolytic activity of the papain type in dough. The inhibition of proteolysis was found to be directly proportional to the amount of flour treated with oxygen. When only the papain was treated with oxygen, there was practically no diminution in proteolytic effect. Baker and Mize (1939) state that in the absence of both yeast fermentation and mechanical motion bromate has little apparent action and that one of the functions of a sponge is the activation of the bromate by the motion which the fermentation produces.

Swanson and Dines (1939) in some work on the wheat-meal-time fermentation test found that since the "time" on flour is longer than on wheat meal, it would appear that the location of the protease is not in the endosperm. They also found that the addition of bran material to the flour increases instead of shortens the "time" although the bran has presumably more proteinase.

Thus it can be seen that there is considerable confusion in the literature, and that not all chemists are agreed that the effect of improvers such as bromate, iodate, Agene, ascorbic acid, and metavanadate is to be attributed to their effect in inhibiting the proteolytic enzymes, however attractive this theory may be.

Since the importance of the proteinase of flour itself has not been clearly demonstrated, and we do not know much concerning its amount or effect on the fermentation of a normal flour, a great many of the



experimental facts brought to bear on the subject have been adapted to the flour-improvement problem in reasoning by analogy as follows rather than by direct evidence. Bromate inhibits the activity of papain; HCN and SH compounds activate the enzyme. Therefore, since flour reacts in a similar way, it must contain a proteolytic enzyme of the papain type which exerts great influence. When too much of the enzyme is present, bromate inhibits the enzyme and greater gas retention and better bread are the result.

It is a foregone conclusion that in any living thing like wheat an enzyme capable of hydrolyzing the proteins under certain conditions would be present. The greatest concentration of this enzyme is probably in the aleurone layer, bran, or germ. On autolysis of the flour itself or in following the fermentation of a dough, the increases in the carboxyl or amino group are not large enough to indicate any considerable hydrolysis to amino acids. It might be argued that perhaps such degradation of the gluten to amino acids would not be necessary and that a partial disaggregation of the proteins as the first step of proteolysis would cause as much ill effect to the breadmaking quality of flours. As Bungenberg de Jong (1938) points out, one of the characteristics of such a disintegration is the quick reaction. In a flour dough an appreciable weakening of the gluten takes place only after some hours. Also, too large an amount of reductant is needed to activate the small amount of proteinase present. Furthermore, even when the proteinase is destroyed by heat an increase in soluble nitrogen is found on the addition of reducing substances.

When germ is added to flour in any appreciable amount it causes a pronounced weakening and this, we found, was due to its content of glutathione. Certain chemists have assumed that the germ acted in this manner because of its content of proteolytic enzymes or because its glutathione activated the proteolytic enzymes of the flour, and both these facts are taken as a support of the proteolytic theory. Flohil's interpretation of his experiments (1936) have supported Jørgensen's theory. Flohil believes that germ exerts its harmful effect because of its proteolytic enzymes. We have always believed that glutathione and other SH compounds acted mainly *per se* on the gluten in case of sound normal flour. The action of such compounds as glutathione, thioglycollic acid, and hydrogen sulfide is too rapid to be attributed solely to enzyme activation. Furthermore, if such compounds acted only as activators of a proteinase, the dough should become more sticky and soft as the time of fermentation increased. As Geddes (1930) has shown, the harmful effect of germ cannot be ascribed to a proteinase added with the germ since the detrimental effect progressively decreased with increasing fermentation time. We have found

that by boiling the water extract of fresh germ for 10 to 15 minutes (which should kill or at least attenuate the activity of the enzyme) the effect was practically as bad as the original unboiled extract. Of course, boiling cannot be continued to the place where glutathione is oxidized or decomposed.

It would seem to us very probable that glutathione acts for the most part directly on the gluten and that the reason that flour-germ mixtures bake better as the fermentation time increases is simply that the glutathione becomes oxidized during the course of the fermentation, consequently lessening its damaging effect.

It was formerly thought that improvers were all oxidizing agents and that damaging results to flour quality were found only with reducing agents. There are, however, certain easily reversible oxidation-reduction systems where either the oxidized or reduced form may be used to cause an improvement. Ascorbic acid-dehydroascorbic acid is one example of this and we have encountered several others.

Since the whole problem of flour improvement was so imperfectly understood in our own minds, we attempted in the beginning to eliminate as many constituents of the flour as we could. Many of the experiments are far from conclusive, but we are including some of them, believing they might be of help to other workers in this field.

### **The Influence of Oxidizing Agents on the Lipids**

Various publications from this laboratory have been concerned with the lipids of flour and wheat germ (Sullivan and Near, 1933; Sullivan, Near and Foley, 1936; Sullivan and Bailey, 1936; Sullivan and Howe, 1938).

When flour is stored under conditions of high moisture and temperature, it may go out of condition and bake very poorly. The unsaturated fatty acids which result on hydrolysis become oxidized and are responsible for the "short" gluten, poor volume, and grain. A flour which is overoxidized by chemicals also gives an open grain and a poor volume. The overoxidization in each case, however, has a different mechanism and although some effects are similar, there are decided differences.

It has also been our experience that the ether extraction of certain flours, particularly our hard spring wheat flours, gives a tough gluten, a tough dough, and poor volume in the bread. The ether extract of the flour will bring it back to normal but the ether extract of germ, lecithin, cottonseed oil, and many other shortenings, though they give better handling characteristics to the dough, will not restore the flour to its normal condition.

Bromate and iodate both give a good response with an ether ex-

tracted flour, although the response is not so large as with the unextracted sample. This is true of the ordinary sponge or straight dough as well as a hearth bread method using a long sponge time, braking the dough, and allowing it to stand on peel for several hours. An ether extracted flour still contains some lipids, but the bromate response would lead one to believe that the lipids are not primarily responsible for the beneficial effect of these oxidizing agents. It was of interest, however, to check this further.

Doughs were made with the untreated and the bromated or iodated flour. After fermentation the gluten was washed from the doughs. The *gluten* from bromated doughs is softer than the gluten from the untreated flour, though the *dough* from the treated flour is shorter than that of the untreated sample. The glutes were analyzed and it was found that while their percentage of nitrogen did not change, their lipid contents (alcohol-ether extract)<sup>1</sup> were significantly different. The glutes from the bromated doughs had less lipid than the gluten from untreated doughs. The gluten from the iodated dough was shorter than those from the bromated or untreated dough. The gluten washed from the iodated dough had still less lipids than the bromated glutes. For example, the dough from the untreated flour showed a lipid content of its gluten of 6%, the gluten from the bromated flour 5%, and the gluten from the iodated dough 4%. The amounts were different depending upon the formula and the time of fermentation. Loss in lipid was greater when no sugar was used in the formula than when yeast, salt, and sugar were used. This loss of lipids in the gluten on treatment with bromate and iodate has been observed on many different crop years and with different types of flour. The longer the fermentation, the greater the loss. We do not know as yet the full explanation of these observations.

It is interesting to note that corresponding to this loss of total lipid on the gluten there is a great loss in nitrogen content of the lipid. The percentage decreases from 1.639% nitrogen on the lipid from the gluten of the untreated flour to 0.02% on the gluten lipid from the sample treated with  $\text{KIO}_3$  as shown in Table II. Accompanying this loss of nitrogen there was also a loss of phosphorus, the weights of the yellow precipitate decreasing from 0.0918g to 0.0777g. These results were obtained with an 18 hour fermentation.

It is generally recognized that lecithin improves baking quality by making the doughs more mellow. Assuming that the loss of lipid is due to the oxidation of lecithin by the  $\text{KBrO}_3$  or  $\text{KIO}_3$ , then the

<sup>1</sup> The sample to be analyzed (in this case the dried gluten) is weighed in an alundum cylinder and refluxed for 2 hours with 95% alcohol, then for 3 hours with ethyl ether. The combined extracts are evaporated almost to dryness (care must be taken at this stage), taken up in ether, filtered through a Bertrand filter packed with asbestos washed previously with water, alcohol, and ether and dried to constant weight *in vacuo* under 100° C.

TABLE II  
EFFECT OF BROMATE ON GLUTEN

Formula	Time	Gluten recovered from dough	Lipid of gluten	Nitrogen of lipid	Phosphorus of lipid
		%	%	%	%
Flour, water	30 min.	13.2	—	—	—
Flour, water	5 hrs.	13.2	—	—	—
2% yeast, 2% salt, 2% sugar	5 hrs.	12.3	—	—	—
2% yeast, 2% salt, 2% sugar, bromate	5 hrs.	12.1	—	—	—
Flour, water	16 hrs.	12.6	—	—	—
Flour, water, bromate	16 hrs.	12.7	—	—	—
2.5% salt, 1% sugar, 0.25% yeast	18 hrs.	11.2	6.32	1.639	0.639
2.5% salt, 1% sugar, 0.25% yeast, bromate	18 hrs.	11.4	5.35	0.729	0.631
2.5% salt, 1% sugar, 0.25% yeast, iodate	18 hrs.	10.8	4.97	0.020	0.609

removal of lecithin whether by ether extraction or oxidation might tighten up the dough and cause an increase in gas retention. Therefore, an improvement in volume by the addition of  $\text{KBrO}_3$  or  $\text{KIO}_3$  would result.

It has been previously stated that the ether extract of germ will not restore ether extracted flour to its original baking status. On the other hand, the ether extract of flour when added to the ether extracted product will return to the flour the original baking characteristics. The fats from both the germ and the flour have been investigated and one of the marked differences between the two fats is that flour fat will give a positive test for loosely bound sulfur. This test is completely negative on the germ fat. This sulfur compound is found in the phosphatide fraction of the fat. The phosphatide fraction comprises 25% of the flour fat and 4.5% of the germ fat. This fraction was separated by repeated precipitations with acetone. Table III

TABLE III  
COMPARISON OF FLOUR AND GERM PHOSPHATIDE FRACTION

	Flour	Germ
% ether extract precipitated by acetone	25.0	4.5
% fatty acids	46.2	—
% nitrogen	4.28	1.211
% phosphorus	1.33	2.21
% sulfur	0.7952	Negative
% sugar as glucose	2.10	1.77
Ratio N : P	7.1 : 1	1.2 : 1
Biuret test	Positive	Negative
Millon's test	Positive	Negative
Xanthoproteic test	Positive	Negative

shows a comparison of the flour and germ phosphatide fraction. Our work would indicate that the phosphatides of lecithin and cephalin are linked to a sulfur-containing peptide. This fraction has a nitrogen content of about 13% and sulfur content of approximately  $2\frac{1}{2}\%$ .<sup>2</sup>

Polarographic results on the lipid material from the gluten do not show the presence of any reducing substance. Likewise, the unextracted and the ether extracted flours give practically the same curves on the polarograph. These results will be reported later.

Our results have indicated that, although the lipid or lipid protein complex of the gluten is important and affected by the addition of oxidizing agents, the influences on the lipid complex by such chemicals as bromate, iodate, or Agene are not primarily responsible for the improving action of oxidizing agents on flour.

### Influence of Improvers on Gassing Power and Diastatic Activity

Many experiments have been done on different flours using improvers such as bromate, iodate, and Agene to see if the gassing power of a flour was affected in any significant way. Small differences in the rate of gassing power are observed but nothing of great significance. In Table IV results are given using the pressuremeter with 10 g. of

TABLE IV  
EFFECT OF POTASSIUM IODATE AND POTASSIUM BROMATE ON GASSING POWER

	Hours					
	1	2	3	4	5	6
	<i>Millimeters of mercury</i>					
No treatment	81	214	362	431	471	502
0.002% KIO <sub>3</sub>	80	214	364	426	462	495
0.004% KIO <sub>3</sub>	80	217	363	426	463	491
0.006% KIO <sub>3</sub>	83	215	362	426	462	494
No treatment	87	220	371	437	476	506
0.002% KBrO <sub>3</sub>	84	226	370	443	487	515
0.004% KBrO <sub>3</sub>	84	221	372	444	486	515
0.006% KBrO <sub>3</sub>	85	217	370	442	484	514

flour (at 13.5% moisture basis) and 7.0 ml. of yeast solution containing 0.3 g. yeast. A short patent (unbleached and treated with 0.1% malt) was employed. The flour was tested using three different amounts of potassium bromate and potassium iodate. No significant difference was observed except for a tendency of the iodate to give slightly lower results beginning with the fourth hour, and the bromate a slightly greater increase in gas production beginning with the fourth hour as compared with the untreated sample. Table V, using an

<sup>2</sup> After this paper was written an article by Balls and Hale appeared in this journal (17: 243-245, 1940) on this same subject.

TABLE V  
EFFECT OF POTASSIUM BROMATE AND POTASSIUM IODATE ON GASSING POWER

Treatment	Hours					
	1	2	3	4	5	6
	<i>Millimeters of mercury</i>					
0.004% KBrO <sub>3</sub>	95	210	339	390	418	450
0.004% KIO <sub>3</sub>	95	209	335	374	401	421

unbleached unmalted straight treated with an equal amount of bromate and iodate, gives a further illustration of the relatively lower gas production with iodate. The same formula was used as given for Table IV. Pressuremeter results using 1% yeast and 3% salt with an untreated short patent and the same flour bromated with 0.004% and 0.016% showed no difference that could be recognized by the pressure readings as illustrated in Table VI. A high percentage of salt, of course, depresses the gassing power.

TABLE VI  
EFFECT OF POTASSIUM BROMATE ON GASSING POWER USING 1% YEAST AND 3% SALT

	Hours					
	1	2	3	4	5	6
	<i>Millimeters of mercury</i>					
Untreated	14	48	87	128	169	208
0.004% KBrO <sub>3</sub>	15	46	87	125	169	202
0.016% KBrO <sub>3</sub>	15	43	83	127	163	200

On the other hand, Figure 1 shows that KBrO<sub>3</sub> under certain circumstances can affect the rate of gas production. A short patent was used with a formula of 1% yeast and 3% salt in order to slow up the fermentation so that gas production could be measured over a relatively long period. Results were obtained on the fermentograph and are corrected for loss by diffusion. It will be noted that all dosages of bromate from 0.004% to 0.012% KBrO<sub>3</sub> definitely depress the CO<sub>2</sub> production as compared with the untreated dough. The lowest amount of bromate has the greatest depressing effect. Figure 2 shows the same material plotted in a different way. In graphing the CO<sub>2</sub> per hour against the hours of fermentation the very characteristic dip is noticed in the fifth hour on this formula. Similar experiments using 3% yeast and 1% salt do not show any significant difference between the gassing power of the untreated and bromated flour. If anything, the bromated dough may give in the later hours slightly more gas.

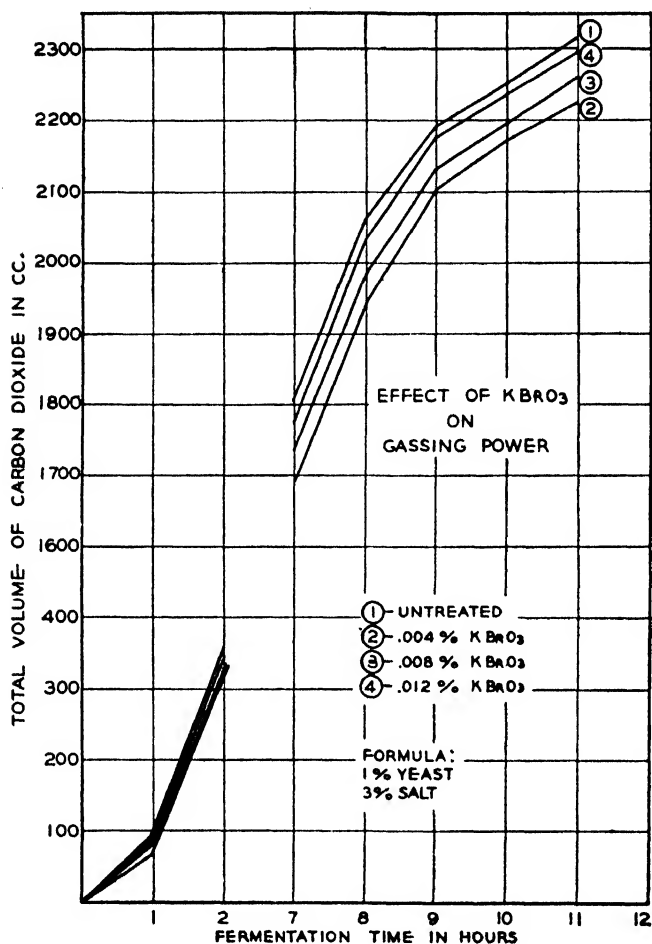


Fig. 1.

The effect of Agene on the gassing power of a spring wheat clear as measured by the pressuremeter is given in Table VII. The usual

TABLE VII

EFFECT OF AGENE ON THE GASSING POWER OF A SPRING WHEAT CLEAR

Clear flour	Hours					
	1	2	3	4	5	6
	<i>Millimeters of mercury</i>					
Unmalted, unbleached	87	211	267	296	319	341
Unmalted, unbleached + 0.2% malt	87	211	309	344	376	394
Unmalted, unbleached + 0.5% malt	88	217	342	380	408	436
Unmalted, unbleached + 1.0% malt	87	214	350	403	438	471
Unmalted, bleached, 10 g. agene	87	210	269	298	323	342
Unmalted, bleached + 0.2% malt	88	211	312	350	380	400
Unmalted, bleached + 0.5% malt	90	221	349	383	410	437
Unmalted, bleached + 1.0% malt	89	218	357	410	444	474

3% of yeast and no salt were employed. As will be noted in Table VII, no marked difference can be detected on either the unmalted or malted samples when bleached with Agene. Agene can modify to a slight extent the rate of gas production, however, when the latter is measured by more sensitive means.

Hence, depending on the formula, particularly the percentage of salt and yeast and the amount of treatment, bromate gave a little greater or a little less gas than untreated flour. Iodate gave slightly less gas. Agene showed no marked effect. While the rate of gas production might be changed slightly depending on the formula and

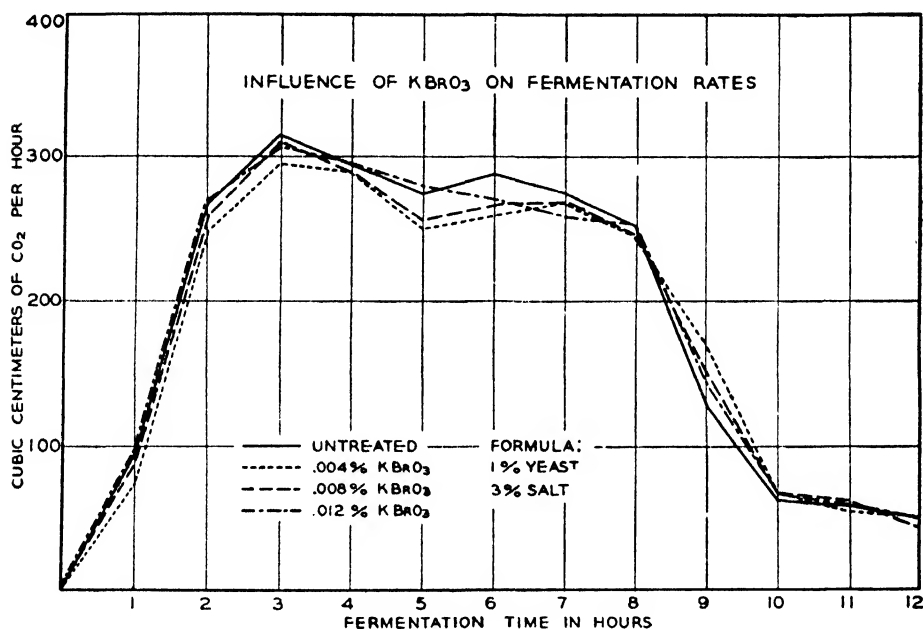


Fig. 2.

fermentation time, the beneficial effect of most of the improvers cannot be explained by any significant effect on the diastatic enzymes. Likewise, the measurement of the maltose value itself on an untreated flour and the same flour treated with Agene, bromate, iodate, metavanadate, and ascorbic acid shows no noticeable difference in the number of units.

Then it was thought that bromate, for example, might conceivably exert a catalytic effect on alpha amylase. The following experiment was tried. Fifty grams of malted wheat flour was made up to 250 cc., centrifuged, and the centrifugate heated for 15 minutes at 70° C. This treatment should kill most of the beta-amylase, according to Ohlson. A spring-wheat unbleached straight was baked alone, with 10 cc. of the alpha-amylase preparation, with 10 cc. of the alpha-



amylase preparation and 0.0047% potassium bromate, and with 0.0047% potassium bromate alone. Results are given in Table VIII. Apparently bromate under these conditions had no effect on the alpha-amylase activity.

TABLE VIII  
EFFECT OF BROMATE ON ALPHA-AMYLASE

	Dough quality	Volume	Grain and texture	Color
No treatment, unbleached flour	Good, elastic	95	Fair	95
10 cc. $\alpha$ amylase	V.G., elastic	97	Good	96
10 cc. $\alpha$ amylase and 0.0047% $\text{KBrO}_3$	V.G., elastic	109½	V.G.	97c
0.0047% $\text{KBrO}_3$	V.G., elastic	110	V.G.	97

### Influence of Starch, Sugars, and Fermentation

Since improvers did not seem to have any significant effect on the diastatic enzymes it was of interest to see if the sugars or the starch fraction could explain, in part at least, the action of such improvers as potassium bromate.

First of all, the amount of potassium bromate used up at the various stages of a straight dough was measured by titration with sodium thiosulfate. It was found that over half of the bromate was used immediately upon mixing the dough. This is probably due to the liberation of glutathione or other reducing substances from the yeast. A comparatively small amount of bromate was used up during the three hours of fermentation. The bread was taken from the oven and it was found that no test for bromate could be obtained although 32.9% bromate was present when the loaf went to the oven. This is illustrated below.

	Percent of total $\text{KBrO}_3$ reduced
From mixer	54.3
During fermentation	12.8
In oven 15 minutes	32.9

It appears that heat is necessary to speed up the reaction which causes the reduction of bromate.

Because of the rapid disappearance of bromate from the dough in the oven, we thought that possibly the  $\text{KBrO}_3$  was oxidizing the sugars present. We made the following series of titrations using maltose and sucrose and titrating with the  $\text{Na}_2\text{S}_2\text{O}_3$ .

Maltose and 5 cc. 0.01N $\text{KBrO}_3$ at pH 5.58
Unheated—required 5.00 cc. of 0.01N $\text{Na}_2\text{S}_2\text{O}_3$
Heated 30'—required 2.70 cc. of 0.01N $\text{Na}_2\text{S}_2\text{O}_3$
Sucrose and 5 cc. 0.01N $\text{KBrO}_3$ at pH 5.58
Unheated—required 5.00 cc. of 0.01N $\text{Na}_2\text{S}_2\text{O}_3$
Heated 30'—required 3.75 cc. of 0.01N $\text{Na}_2\text{S}_2\text{O}_3$

We then made up two doughs with and without  $\text{KBrO}_3$  and determined the amount of sucrose and maltose remaining in the bread. The following results were obtained:

	% sucrose remaining in bread	% maltose remaining in bread
Bromated dough	0.47	2.51
Regular dough	0.52	2.41

It is evident from this experiment that the bread from the bromated dough contains about the same amounts of sucrose and maltose as the bread from the untreated dough. However, it is interesting to note that bromated and iodated doughs are invariably darker in crust color than the unoxidized flour, while Agene treatment has the tendency to produce a paler crust than the untreated flour.

The following experiment was conducted to determine the extent of the starch influence. Starch and gluten were separated by washing, and the starch fraction was obtained by centrifuging the water suspension. This fraction was fermented 3 hours with yeast, salt, and  $\text{KBrO}_3$ . An amount of bromate equivalent to 10.8 cc. of 0.01N  $\text{Na}_2\text{S}_2\text{O}_3$  was used.

Starch fermented 3 hours	5.08 cc. 0.01N $\text{KBrO}_3$ reduced
Starch fermented 3 hours, 30 minutes in oven	6.15 cc. 0.01N $\text{KBrO}_3$ reduced
Dough fermented 3 hours	5.95 cc. 0.01N $\text{KBrO}_3$ reduced
Dough fermented 3 hours, 30 minutes in oven	10.80 cc. 0.01N $\text{KBrO}_3$ reduced

From these experiments it can be seen that there is something present in the dough not found in the starch that reduces all the  $\text{KBrO}_3$  when heat is applied. This is probably found in gluten. The starch fraction, however, like the lipids, exerts a secondary effect.

The following experiment may also be of some interest as to what occurs during fermentation. Sublimed sulfur (0.33%) was added to the ingredients of a straight dough. The dough became soft and sticky and resembled a dough which had been treated with glutathione or excess amounts of cysteine. An unmistakable odor of  $\text{H}_2\text{S}$  was emitted by the dough during fermentation. This softening of the dough and the  $\text{H}_2\text{S}$  odor are indications that a reducing medium exists in the dough of such strength that it is able to reduce sulfur to  $\text{H}_2\text{S}$ , which in turn exerts the characteristic effect of the SH group on the gluten.

The following experiment is of interest in this connection. A short patent was employed in a straight-dough process. Various amounts of sublimed sulfur were tried as illustrated in Table IX. It can be seen that here, too, 0.1% sulfur (based on the flour weight) was very damaging. The dough was short and sticky and the resultant bread had poor volume and a coarse open grain. The bread was similar

TABLE IX  
EFFECT OF VARIOUS AMOUNTS OF SUBLIMED SULFUR

Flour	Treatment	Feeling of dough	Loaf volume	Grain and texture
Short patent	None	Very good, elastic	100%	Very good
Short patent	0.05% S	Good, elastic, sl. soft	96%	Good
Short patent	0.10% S	Fair, elastic, very soft	80%	Poor
Short patent	0.17% S	Poor, very soft, sticky	54%	Very poor
Short patent	0.33% S	Soft and sticky, could not be handled	—	—

to loaves made from flour to which germ or glutathione had been added. Another experiment was tried using sulfur with and without bromate as illustrated in Table X. Bromate improved the loaf considerably.

TABLE X  
EFFECTS OF SULFUR WITH AND WITHOUT BROMATE

Flour	Treatment	Feeling of dough	Loaf volume	Grain and texture
Short patent	None	Very good, elastic	100%	Very good
Short patent	0.1% S	Fair, short, very soft	82%	Poor, coarse
Short patent	0.1% S	Good, sl. short	97%	Good plus
Short patent	0.0045% KBrO <sub>3</sub>			
Short patent	0.0045% KBrO <sub>3</sub>	Good, sl. tight, sl. short	93%	Fair, sl. open, tough
Short patent	0.13% cystine	Fair, sl. soft	96%	Fair, open, tough

The same amount of bromate when used in the dough without the sulfur addition showed over-oxidation of the flour. Large amounts of cystine can also be reduced, although with less effect than with the same percentage of sulfur. It is of interest in connection with sulfur that flour-water curves on the farinograph using 0.33% sulfur showed only a slight softening, indicating that time or conditions of fermentation are necessary for the reduction.

### Influence of Gluten

Since neither the lipids, starch, sugars, nor diastatic enzymes appeared to be primarily responsible for the beneficial effect of certain oxidizing agents, everything seems to point to the gluten. Many factors influence the response of flour to oxidation such as climate and other growing conditions of the wheat, but it is frequently the case that the higher the protein content of the wheat the greater the bromate response of its flours and the more treatment the flour requires for best baking results. This indicates that the oxidizing effect may be primarily due to the gluten.

It was mentioned earlier that the action of certain compounds containing a sulfhydryl group was very rapid. For example 110 g. of wet gluten from 240 g. of flour was ground with 0.1 cc. of thioglycollic acid. The gluten disintegrated rapidly and became almost completely peptized. Cysteine and glutathione also cause an extreme softening and liquefaction of the gluten. In our opinion, the action of these SH compounds is not on any proteolytic enzyme because one would expect the latter to have been washed away, to some extent at least, in recovering the gluten. Furthermore, the amounts of these sulfhydryl compounds necessary to damage the bake are larger than one would expect if they acted merely as activators of the proteolytic enzyme. It is difficult to prove definitely that the activation of the proteolytic enzymes is a secondary effect until we have a better way of measuring proteolysis. The difficulty is greater because proteolytic enzymes of the papain type contain a sulfur linkage like the gluten proteins.

In order to obtain some direct proof that a reducing group could be liberated from the gluten, the gluten was washed from 30 g. of a spring wheat clear having 16.5% protein. The wet gluten was cut in very fine pieces and 2 cc. dichlorophenolindophenol (0.1 g. to 200 cc.) and 98 cc. of a buffer solution pH 5.59 added. A blank was made up using the same quantities of indicator and buffer. On heating on the steam bath, the gluten flask became colorless in about an hour while the blank remained the same original shade of blue. Different experiments of this nature showed the presence of a reducing substance on heating or on standing a considerable time, but the experiments were unsatisfactory and could not be made quantitative because only the surface of the gluten was affected.

The following experiment may be of some interest. A straight grade flour (unbleached and unmalted) having 0.46% ash and 14.10% protein (calculated at 13.5% moisture) was doughed up using ingredients given below:

Treatment	Yeast %	Salt %	Sugar %
1. None	2	2	None
2. 0.005% KBrO <sub>3</sub>	2	2	None
3. None	2	2	5
4. 0.005% KBrO <sub>3</sub>	2	2	5
5. None	None	None	None

Dough No. 5 (flour and water) was washed out after standing 30 minutes. The other doughs were handled as follows: 1st punch, 1 hour 45 minutes; 2nd punch, 30 minutes; to bench, 20 minutes; to oven, 1 hour 25 minutes later. After this total of four hours of fermentation, the glutens were washed out and analyzed. The glutens

washed from a fermented dough start out being short, then milky, and always end up shorter than the original flour-water gluten. The bromated doughs are always tougher or shorter than the unbromated, but the *gluten* washed from these bromated doughs is more extensible than the gluten from the untreated dough. The percentage of gluten recovered and the analyses of the glutes are given in the table below.

Experiment No.	Dry gluten from doughs %	Moisture %	Protein (N $\times$ 5.7) %	Lipid %	Ash %
1	12.5	1.43	90.10	6.75	.34
2	12.4	1.53	90.50	5.37	.29
3	12.2	1.60	91.00	6.92	.25
4	12.3	1.80	90.75	6.00	.25
5	13.3	1.51	89.10	7.01	.36

As has been mentioned before, some of the gluten cannot be recovered after fermentation although that which can be recovered is, as a rule, in good shape unless the fermentation time is very long. The longer the fermentation time, the more difficult it is to recover the gluten because of its extreme shortness.

Because the amount of starch held by these glutes was so small, it was not measured. Better than 7% of the total gluten was lost during fermentation. As pointed out under lipids, a greater loss of lipids was encountered in all the fermented doughs than in the case of the flour-water dough which stood only 30 minutes (Experiment No. 5). Also, the bromated dough lost more lipid than the untreated dough and the dough containing no sugar lost more lipids than the 5% sugar dough. There is no significant difference in the protein content of the glutes.

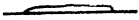

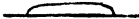
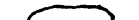


The experiments thus show that both the chemical analysis and physical properties of a gluten which has undergone fermentation are changed.

### **Influence of Bakery Machinery**

The amelioration of overoxidation by apparently purely physical means is most peculiar. Bromate and iodate, when used in more than optimum amounts in an ordinary formula, have a tendency toward shortening or lessening the elasticity of a dough. Freilich and Frey (1939) have shown that an overtreated dough showing signs of shortness and age can be brought back by remixing. We have long observed that the shortness and age shown in a treated flour can also be overcome in part by putting the dough through a dough brake several times.

A short patent (0.39% ash, 12.80% protein) was baked with no bromate and 0.0045% bromate using the following formula: yeast, 2%;

salt, 2%; sugar, 4%; malt, 1%; dry skim milk, 3%; lard, 3%. Absorption was 62%. First punch was given after 1 hour and 45 minutes, second punch after 30 minutes. The doughs were left 25 minutes and then panned or broke. After molding or braking, the doughs were placed on boards and allowed to remain 19 hours. The appearance after this time is roughly illustrated below.

	Treatment	Appearance
1. No $\text{KBrO}_3$	Hand molded	
2. 0.0045% $\text{KBrO}_3$	Hand molded	
3. No $\text{KBrO}_3$	Machine molded	
4. 0.0045% $\text{KBrO}_3$	Machine molded	
5. No $\text{KBrO}_3$	Dough brake 10 times	
6. 0.0045% $\text{KBrO}_3$	Dough brake 10 times	

The largest loaf by far when part of the same dough was baked was No. 6.

The amount of bromate which produces the best loaf on molding and handling, according to the normal punching and fermentation time, is less than the amount that will produce the biggest volume with a brake dough.

The return to normal on remixing or braking of an overtreated dough is difficult to explain other than on the basis that some sort of a redox system is present in the dough or by the purely physical changes in the dough structure resulting from mechanical handling.

### Discussion

Many of the well known flour improvers have outstanding differences in their mechanism of reaction in flour doughs even though the end result of all of them is an improvement in some such factor or combination of factors as absorption, fermentation tolerance, handling characteristics, or the volume, grain, or texture of the finished bread.

As is well known, any given flour which responds to an improver demands a certain critical amount of the improver for any given formula and procedure. Overtreatment can produce worse results than no treatment at all. For best baking results a flour must have a certain definite oxidation potential whether it has obtained it from the wheat or by artificial treatment.

No two flour improvers exert their effect in exactly the same way. For instance, iodate reacts very differently from bromate. The action

of iodate is very rapid. An iodated dough feels tighter immediately after mixing. On the other hand, a bromated dough feels no different from the mixer than the untreated sample. Bromate does not exert its effect in any physical manner until sometime after fermentation has started. An explanation of this fact lies in the different pH requirements for the oxidizing actions of bromate and iodate. It is known that in order to exert its oxidizing effect iodate requires less acidity than bromate. A few experiments as given below using buffer solutions of various pH were done to illustrate this difference in behavior.

To 100 ml. of the buffer solution was added 20 ml. of 0.01*N* potassium bromate or 20 ml. of 0.01*N* potassium iodate together with 10 ml. of 30% potassium iodide. After a time interval (20 to 30 minutes) starch solution was added and the liberated iodine was titrated with 0.01*N* sodium thiosulfate.

pH	Oxidized by KBrO <sub>3</sub>	Oxidized by KIO <sub>3</sub>
4.0	No	—
5.0	No	Yes
5.5	No	Yes
6.0	No	Yes
6.5	No	No

A longer time interval would probably have given the bromate a chance to exert its oxidizing effect. Also, a different reducing substance such as cysteine or glutathione might have been oxidized by bromate at the pH levels of dough. The above tabulation, in any case, shows that potassium iodate acts as an oxidizing agent more readily and at higher pH levels than potassium bromate. This can be illustrated also by the following experiment. A sample of flour was divided and one portion treated with 0.002% KBrO<sub>3</sub> and another with 0.002% KIO<sub>3</sub>. A water extract of each of these flours was made, and KI, H<sub>2</sub>SO<sub>4</sub>, and starch indicator solution added in the proper amounts. In the case of the water extract from the bromated flour, one can obtain a titration with thiosulfate but none can be obtained with the iodated flour. This indicates that the KIO<sub>3</sub> is entirely reduced while the KBrO<sub>3</sub> is not completely reduced.

On the other hand, iodate is similar to bromate in that it will make a dough which has been run through a dough brake, hold gas over a long period on the peel and give a good volume, whereas Agene and ascorbic acid will not. Iodate must always be used in smaller amounts than bromate for the same effect. Vanadate, likewise, gives results similar to bromate and iodate but in still smaller amounts than iodate. Vanadate and iodate when used in a sponge do not give the characteristic appearance of breaking as will the untreated or bromated

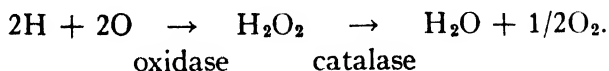
doughs. Copper salts, either cuprous or cupric, will likewise give good results on a long time lean dough but must be used in an amount approximately double that of bromate.

Agene and bromate act in a different manner also in that Agene treatment can often be felt immediately from the mixer and on the washed gluten from a treated flour, whereas bromate cannot except after long standing.

It is known that bromate plus minute amounts of vanadate has a beneficial effect on doughs and enables less bromate to be used. We assume that this is due to the fact that the reaction rate between SH (or a similar reducing group of the protein molecule) and vanadium must be faster than the rate between bromate and the reducing group. It is conceivable that the SH group reduces the vanadium in sodium metavanadate to  $V^{++}$ . The  $V^{++}$  is oxidized to  $V^{++++}$  and the SH reduces the  $V^{++++}$  to  $V^{++}$  again when the cycle may continue until the equilibrium is thrown too far to one side. It is important to remember that the  $SH \rightleftharpoons S-S$  is not an easily reversible system under all conditions. The H ion as a rule is rather easily detached from an SH group.

The explanation of the action of certain chemicals in improving the baking quality of flour hinges on the proof that SH or some similar reducing group is present in the dough. A complete proof of this is needed before the proteolytic theory or our own could be accepted. Assuming the fact that a reducing group is present in the protein and is fairly readily dissociable, the following mechanisms could be offered in explanation of the beneficial effect of certain improvers. Certain other possible theories are also discussed.

Some flours have more of a reducing substance than others. During fermentation the dehydrase of the yeast liberates hydrogen from this substance. The active  $H^+$  is then combined with oxygen which has been activated by an oxidase or a metal (Cu or V) or by some oxidizing agent capable of a steady supply of active oxygen at the pH of dough. Cu or V are able to cause a definite electron transfer which the organic substances present cannot do. The  $H^+$  released from the reducing substance then could form  $H_2O_2$  which would be acted on by catalase to form water and oxygen as follows:



Oxidase is inhibited by bisulfite, HCN, and certain other reducing substances. A flour which responds to  $KBrO_3$ ,  $KIO_3$ , Agene, etc., has a higher content of reducing substances or substances capable of giving up  $H^+$  under influence of dehydrases. A flour not responding



has only a small amount of such  $H^+$  donating substrates. The oxidation reduction process goes on until the  $H^+$  donator is used up in cases where oxygen is supplied by bromate, iodate, or mixing. In the case of Agene the probability is that  $NCl_3$  combines with the oxidases to stop the action at one certain stage, thus preventing the further continuation of the oxidation reduction system.

Another theory may be considered more likely. There are present in gluten certain  $S-H$  groups bound to other protein linkages or  $S-S$  groups capable of reduction to  $SH$ . During fermentation some of these sulfhydryl groups dissociate and can be oxidized by oxygen from the air or from oxidizing agents such as bromate or iodate. The rate of oxidation is dependent on the degree of dissociation of the  $SH$  groups from the gluten and the oxidation potential of the fermentation. Certain metals such as  $Cu$  can form mercaptides and this system as well as the  $2SH \rightleftharpoons S-S + 2H$  system is in a certain measure reversible. Many other systems such as ascorbic-dehydroascorbic acid act as oxygen carriers. Agene, no doubt, oxidizes the  $SH$  more or less irreversibly.

The alteration of the gas-retaining capacity of a dough by purely physical means such as mixing, punching, molding, and braking as well as by certain chemicals is caused by a change in the protein structure. Our work has given strong indications that some change in the labile sulfur linkages of the gluten can explain a number of the problems of flour chemistry. Such reactions are responsible for the change in the physical characteristics of certain doughs and the improvement given by oxidizing agents or oxidation-reduction systems.

If we assume that the gluten strands are coiled fibrils, it is conceivable that a main or side chain containing  $R-S-S-R$  may be acted on by oxygen and water (or agents capable of supplying oxygen) to form  $R(SO)_2R$ ,  $RSO_3H$  or  $RSH$  etc., whereby the sulfur linkage would be altered or broken. If a scission of the molecule should take place, it would result in shorter strands, less extensibility and greater "shortness." Similarly certain reducing agents might attack the sulfur linkage not causing a hydrolytic cleavage but only the uncoiling of the long fibrils producing greater softness and extensibility but less elasticity. It is possible to imagine that in the first instance the broken fibrils may be knitted together or aligned to some extent by purely physical means such as braking a dough. The effect of reducing agents is usually a greater softness as well as shortness of the dough. The softness or too great extensibility may be overcome to some extent by punching or molding since such physical procedures would coil back the untwisted strands if too much damage had not been done to the sulfur linkage of the protein molecule.

Needless to say, such a theory is difficult to prove because the sulfur compounds are not present in a free state and therefore their isolation is difficult. Even on pure compounds such as cystine, the mechanism of its oxidation and reduction is very imperfectly understood. However, the effect of oxidizing and reducing agents on flour seems to be explained best with the facts available at present on the basis of their direct effect on the sulfur linkage of the flour proteins.

### Conclusion

Several experiments bearing on the action of oxidizing and reducing agents on flour and in dough have been described. A few theories have been presented as possible explanations. All the work indicates that changes in the sulfur linkages of the gluten proteins are responsible for many of the effects described.

### Literature Cited

- Baker, J. C., and Mize, M. D.  
1937 Mixing doughs in vacuum and in the presence of various gases. *Cereal Chem.* **14**: 721-724.  
1939<sup>\*</sup> The reaction of mechanical stirring to sponge doughs. *Cereal Chem.* **16**: 294-295.
- Balls, A. K. and Hale, W. S.  
1936 Further studies on the activity of proteinase in flour. *Cereal Chem.* **13**: 656-664.  
1938 The preparation and properties of wheat proteinase. *Cereal Chem.* **15**: 622-628.
- Bungenberg de Jong, H. I.  
1938 Proteins in the flour industry. Symposium on proteins. Amsterdam.
- Flohil, J. T.  
1936 The effect of chemical flour improvers on proteolytic action in relation to the gas retaining capacity of fermenting doughs. *Cereal Chem.* **13**: 675-689.
- Ford, W. P., and Maiden, A. M.  
1938 Effects in dough of glutathione and papain. *J. Soc. Chem. Ind. (Transactions)* **57**: 278.
- Freilich, J. and Frey, C. N.  
1937 The effect of oxygen on proteolytic activity in bread dough. *Ind. Eng. Chem. (News ed.)* **15**: 294.  
1939 Dough oxidation and mixing studies. II. Effects of remixing after fermentation. *Cereal Chem.* **16**: 492-502.
- Geddes, W. F.  
1930 The influence of germ constituents on baking quality and their relation to improvement in flour induced by heat and chemical improvers. *Can. J. Research* **2**: 195-213.
- Jørgensen, Holger  
1935 Ein Beitrag zur Beleuchtung der hemmenden Wirkung von Oxydationsmitteln auf proteolytische Enzymtätigkeit: Über die Natur der Einwirkung von Kaliumbromat und analogen Stoffen auf die Backfähigkeit des Weizenmehles. I. *Biochem. Z.* **280**: 1-37 (abridgement in *Muhlenlab.* **5**: 113-126).  
1938 Entwicklung der Theorie der Bromatwirkung, 1935-1938. *Muhlenlab.* **8**: 201-208.
- Read, J. W. and Haas, L. W.  
- 1939 Studies on the baking quality of flour as affected by certain enzyme actions. VI. Further studies relating to the activation and inhibition of flour proteinase. *Cereal Chem.* **16**: 60-69.

- Sullivan, B., and Bailey, C. H.  
1936 Lipids of the wheat embryo. I. The fatty acids. II. The unsaponifiable fraction. *J. Am. Chem. Soc.* **58**: 383-393.  
— and Howe, M.  
1937 The isolation of glutathione from wheat germ. *J. Am. Chem. Soc.* **59**: 2742.  
1938 Lipids of wheat flour. I. The petroleum ether extract. *Cereal Chem.* **15**: 716-720.  
— — and Schmalz, F. D.  
1936 On the presence of glutathione in wheat germ. *Cereal Chem.* **13**: 665-669.  
— and Near, Cleo  
1933 Change of crude lipids of wheat on storage. *Ind. Eng. Chem.* **25**: 100.  
— and Foley, G. H.  
1936 The role of lipids in relation to flour quality. *Cereal Chem.* **13**: 318-331.  
Swanson, C. O., and Dines, F. T.  
1939 The wheat-meal-time-fermentation test. II. Effect of proteases, protease activators, and protease inhibitors. *Cereal Chem.* **16**: 168-177.

## THE RELATION BETWEEN THE NORMAL FARINOGRAM AND THE BAKING STRENGTH OF WESTERN CANADIAN WHEAT <sup>1</sup>

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(Read at the Annual Meeting, May 1939)

When the Brabender farinograph was introduced on the American continent it was suggested (Brabender, 1934) that the relative strength of different flours could be predicted from measurements of the curves produced by the instrument. More recently the original claims made for the instrument have been modified and Munz and Brabender (1940) now write: "The farinograph is adapted to the measurement of dough plasticity as a function of continuous mixing. This instrument has also proved successful in estimating the capacity of a strong flour to yield doughs of acceptable properties when blended with weak flour. From the characteristics of farinograms, other dough properties, such as the rate of hydration, sensitivity to mixing, and buckiness, can be estimated. The farinograms are of limited value in certain other particulars, however. Thus, they fail to reveal the direction and magnitude of the effect of chemical treatments, or the recovery or tendency of the dough to regain certain of its original properties after excessive mixing." In connection with this quotation it is worth noting that most of the current literature on the farinograph deals with studies which are not primarily concerned with its utility for predicting baking

<sup>1</sup> Contribution from the Grain Research Laboratory, Board of Grain Commissioners for Canada, Winnipeg, Manitoba. Published as Paper No. 178 of the Associate Committee on Grain Research of the National Research Council of Canada and Dominion Department of Agriculture.

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strength. The majority of the papers describe applications of the instrument to such problems as the determination of absorption, calibration of dough mixers, the estimation of proteolytic activity and studies on the effect of starch, dried gluten, milk solids and germ constituents on the colloidal properties of wheat-flour doughs.

In 1935, when the work described in the present paper was started, there were some who believed that measurements of the physical properties of doughs might replace the baking test as a rapid method of assessing baking quality, though published data bearing on this hypothesis were negligible in amount and many cereal technologists did not support the idea. Since that time statistical studies of the relations between the physical properties of doughs and baking characteristics have been made by Markley and Bailey (1939). These workers found that protein content was highly correlated with both flour absorption and dough-development time and that, in a series of 23 spring-wheat flours, the correlation between protein content and loaf volume was much higher than that between the decrease in consistency upon extended mixing in the farinograph and the volume of the test loaves. They also reported that the increase in mobility of doughs upon prolonged mixing was not significantly correlated with the decrease in loaf volume resulting from similar treatment.

Incidental to the routine testing of Western Canadian hard red spring flours, it has been the practice of the Board of Grain Commissioners' Laboratory to make normal farinograms whenever time permitted and a considerable volume of data has been accumulated. In view of the limited information in the literature on the relation between loaf volume and characteristics of the normal farinogram, statistical studies of a portion of these data are now reported in this paper.

In a preliminary investigation, the results obtained with the 300-g. and 50-g. Brabender mixers were compared using five blended flours of graded strength and three dough-consistency levels, namely 500, 600, and 700 Brabender units. This investigation provided information not only on the relative performances of the two sizes of mixers but also on the effects of widely different baking strengths and different dough consistencies on the characteristics of the normal farinograms.

In the major investigation, protein determinations, baking tests, and farinograms were made on a total of 333 samples of Western Canadian hard red spring wheat flours, arranged in four series, each of which was studied separately. This investigation permitted a statistical study of the correlations between flour protein, absorption and loaf volume, on the one hand, and certain measurable characteristics of Brabender curves, on the other.

### Methods

*Baking.*—In the main study, the experimental material comprised long-patent flours experimentally milled from Western Canadian hard red spring wheat, and the samples were baked by the malt-phosphate-bromate formula using the procedure outlined in the A. A. C. C. basic baking test (Geddes, 1934). This formula differs from that of the basic formula in that 0.3% diastatic malt (250° Lintner), 0.1%  $\text{NH}_4\text{H}_2\text{PO}_4$ , and 0.001%  $\text{KBrO}_3$  are added as extra ingredients. The doughs are mixed for three minutes at medium speed in the Hobart mixer equipped with two dough hooks and baked in low-sided tins. Extensive experience in determining the relative strength of Canadian hard red spring wheat involving tests on untreated and only slightly aged experimentally milled flours has shown that it is necessary not only to provide for adequate gas production but also to employ an oxidizing agent to assist in proper development of the gluten. Numerous trials with various baking formulas and procedures (e.g. Aitken and Geddes, 1934) led to the adoption of the above formula for routine testing purposes since it gives wide differentiations between flours; the loaf volumes for commercially grown varieties and the various wheat grades are also in line with their accepted evaluation by Canadian and overseas millers.

*Brabender farinograms.*—Aside from the preliminary study involving comparisons of the 50-g. and 300-g. Brabender mixers, the small mixer was used throughout. Fifty grams of flour (13.5% moisture basis) was mixed at 30°C. for 15 minutes at 600 farinograph units, a preliminary curve being run to determine the required amount of water.

For statistical purposes, it is necessary to secure quantitative values representing the curve characteristics, the most significant of which seemed to be (1) the "dough-development time" or mixing time required to produce a dough of maximum consistency, (2) the decrease in consistency upon overmixing, and (3) the mean width of the curve after maximum dough development. At the time these studies were initiated, no methods for evaluating these curve characteristics had been proposed and the following measurements, as illustrated in Figure 1, were arbitrarily selected.

1. Dough-development angle is the angle between the line drawn from the midpoint of the curve at maximum dough development to where the curve meets the 400-unit line and this latter line.

2. Weakening area is the area in square centimeters as measured by a planimeter, enclosed by a line drawn from the midpoint of the curve at maximum dough development to the end of the curve (15 minutes total mixing time) parallel to the 600-unit line, and from this point

down to the midpoint of the end of the curve and returning to the dough-development point.

3. Mean band width is the total area of the curve (in square centimeters), omitting irregularities, from the point of maximum dough development to the end of the curve (15 minutes total mixing time), divided by the length of the median line of this portion of the curve.

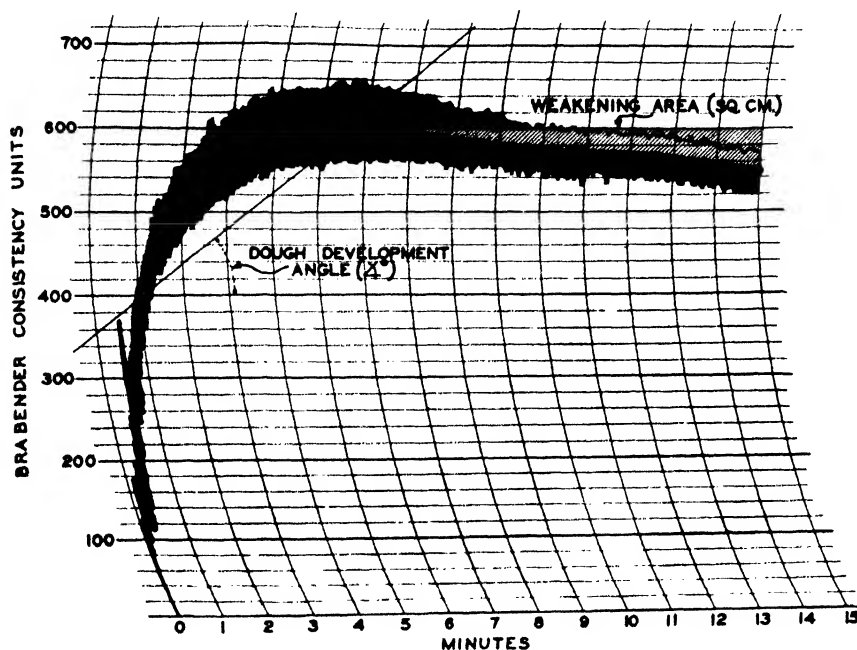


Fig. 1. Normal farinogram, showing the methods employed in securing quantitative measures of dough-development time (dough-development angle), mobility increase upon overmixing (weakening area), and mean band width.

The dough-development angle was originally selected as a measure of dough-development time because it was felt that it could be determined with greater precision than the direct estimation of this value from the farinograms. This choice was unfortunate since such indices of dough-development time for curves made at different mobility levels are not directly comparable and the values for any particular level are subject to error where the midpoint of the band at maximum dough development does not fall precisely on the mobility level selected; moreover the angle is inversely related to dough-development time, and this is somewhat confusing. In the later series of measurements, the dough-development time as read from the farinograph was also recorded and employed in the statistical studies.

#### I. Comparison of Large and Small Mixers—Effect of Absorption and Dough-Consistency Level on Farinograph Curve Characteristics

At the time the farinograph was acquired, the small or 50-g. mixer had just been introduced and the manufacturers recommended that

the large or 300-g. mixer should be used whenever possible; moreover, it was suggested that the "dough consistency" at 500 units, recommended for use in Europe, might not be entirely satisfactory for the stronger flours with which the authors had to deal. Accordingly, before employing the farinograph as a routine testing instrument, a preliminary study was undertaken to determine the relation between the two mixers in regard to curve type and replicability of results and also as an indication of the most desirable consistency level to employ to secure the optimum differentiation between samples.

For this purpose, a suitably graded series of flours was obtained by using a high-protein Canadian spring wheat baker's patent and a low-protein cake flour, together with three blends of these. Farinograms on each of these five flours were made in quadruplicate in both the large and small mixers at dough-consistency levels of 500, 600, and 700 units. Measurements of the curves were made in duplicate and submitted to statistical analysis.

Both mixers readily differentiated between the flours in regard to dough-development time and weakening area. While the two mixers gave different absolute values for the curve characteristics with the same flours, the general trends with changing flour strength were quite similar. The changes in dough-development time and weakening area for the extremes in strength represented in this series were, on the average, slightly higher for the small mixer but the replicability of the tests in the large mixer tended to be somewhat better. It was noteworthy that the flour absorptions required to yield doughs giving the same consistency values in the two mixers differed materially. The mean difference for all consistency levels varied from 0.6% for the weakest flour to 2.8% for the strongest flour. The importance of these differences in relation to the use of the farinograph as a standard method for determining absorption will not be discussed at this time but it should be mentioned that the instruments used in the present study were purchased before it became the standard practice of the manufacturers to calibrate all mixers against a standard machine at 500 units.

In spite of the fact that the larger mixer appeared to be somewhat superior with respect to replicability, it was decided to adopt the small mixer for all routine studies. The replicability and differentiations obtained with the latter were quite adequate for most purposes and it has the added advantage of requiring only one-sixth the quantity of flour per test, which is of particular importance in this laboratory where the size of sample available for study is frequently limited.

The most suitable mobility level to employ in routine studies was not conclusively indicated by the preliminary study. The character

of the curve for any particular flour is materially influenced by the consistency level employed. Very stiff doughs give a short and fairly precise dough-development time, a wide band and a rapid break-down or decrease in consistency upon further mixing. As the consistency is lowered, the curve band becomes narrower and the decrease in consistency much less rapid; at low consistencies strong flours actually give a rising curve for the entire 15-minute mixing period, thus giving a long and indeterminate dough-development time and a negative weakening area. At the 500-unit level, the increase in mobility with extended mixing is relatively slight for strong flours and it is difficult to estimate the point of maximum dough development with accuracy since the curve usually has a zero or very slight negative slope for an appreciable interval of time. The 700-unit consistency gave the greatest range in the mean weakening area for the various flours, but the replicability was in general not as satisfactory as for the curves made at 600-units consistency. On the whole, it appeared that the intermediate level of 600 units would prove most satisfactory for the flours ordinarily tested in the Laboratory, and this level was accordingly used in further investigations reported in this paper.

## II. Relation between Curve Characteristics and Loaf Volume

While there are wide differences in the types of normal farinograms given by strong and weak flours when mixed to the same maximum consistency, it is of interest to determine whether, with flour of the same general type, curve characteristics such as development time, width of curve, and decrease in consistency upon overmixing are related to baking strength (as indicated by loaf volume determined by the malt-phosphate-bromate baking formula), or reflect other properties not related to baking strength. It is also of interest to ascertain the relative degrees of association between these individual curve characteristics and loaf volume.

It is well known that oxidizing agents, such as potassium bromate, have pronounced effects on the baking properties of flours, yet such flours yield normal farinograms essentially similar to the corresponding untreated samples. Since commercial flours may vary widely in the extent of treatment with maturing agents, no general relation between the baking results and curve characteristics of such flours can be expected. Such relations might, however, be found with samples of untreated flours of similar history and such samples provide experimental material most favorable to the farinograph for determining its possible value as an index of baking strength. Flours experimentally milled from different grades of Canadian wheat are, therefore, very suitable for investigating the usefulness of the farinograph in this



connection. They are classed on the world's markets as strong and medium-strong flours and the various grades from which they are milled exhibit differences in average strength which are universally recognized by the domestic and foreign trade. Such general commercial evaluations are useful in a study of this kind, where the integrity of the experimental baking test as a standard of reference may be questioned by some workers.

The relations between farinograph and baking data were studied for four series of untreated long-patent experimentally milled flours of approximately equal extraction representing: (1) 215 samples of wheat cargoes of the 1935-36 crop year; (2) 48 samples of plant breeders' varieties of the 1935 crop; (3) 30 samples of Winnipeg inspection office averages for different grades representing three crops, 1936, 1937, and 1938; and (4) a frosted wheat series representing 40 samples of No. 3 and No. 4 Northern wheat collected for an investigation of the effect of frost damage on milling and baking characteristics. (See Malloch, Geddes, Larmour, and McCalla, 1937.)

*Cargo series.*—The minimal, maximal, and mean values for flour protein, loaf volume, absorption at 600 Brabender units, and farinogram measurements are recorded in Table I together with the standard deviations of the individual values; the results of variance analyses, expressing the significance of the differences in the respective mean values for the three grades, are also summarized by the *F* values and their 5% points.

The differences in mean protein content and loaf volume for the three grades are highly significant while the absorption values are closely similar. The means for dough-development angle follow essentially the same trend as those for loaf volume and protein content but the mean weakening areas for the various grades are not significantly different and those for band width differ but slightly and do not show a consistent trend with grade.

The simple correlation coefficients given in Table II show that loaf volume, dough-development angle, and weakening area are significantly correlated with protein content. The correlation between protein and loaf volume (+.903) is significantly higher than the correlations between protein content and dough-development angle (−.735) and protein content and weakening area (−.652). In interpreting the correlations involving dough-development angle, it should be recalled that this measure is inversely related to dough-development time. The correlation between loaf volume and dough-development angle (−.699) and that between loaf volume and weakening area (−.619) are not significantly different but are significantly lower than the correlation between loaf volume and protein content (+.903).

TABLE I  
STATISTICAL CONSTANTS FOR FLOUR PROTEIN, ABSORPTION, AND FARINOGRAPH  
MEASUREMENTS ON CARGO SAMPLES

	No. 1 Northern 82 samples	No. 2 Northern 60 samples	No. 3 Northern 73 samples	All grades 215 samples
MINIMA				
Flour protein, %	12.7	11.0	10.5	10.5
Loaf volume, cc.	852	742	558	558
Absorption (at 600 B.U.), %	55.0	54.6	55.2	54.6
Dough-development angle degree	26.5	22.0	23.5	22.0
Weakening area, sq. cm.	1.1	0.2	0.2	0.2
Mean band width, cm.	0.93	0.50	0.82	0.50
MAXIMA				
Flour protein, %	14.5	15.1	14.0	15.1
Loaf volume, cc.	1030	992	940	1030
Absorption (at 600 B.U.), %	60.6	61.6	61.2	61.6
Dough-development angle degree	47.0	50.0	69.5	69.5
Weakening area, sq. cm.	7.3	6.0	12.0	12.0
Mean band width, cm.	2.30	2.80	1.43	2.80
MEANS				
Flour protein, %	13.30	13.04	12.08	12.81
Loaf volume, cc.	932.4	877.0	744.0	853.0
Absorption (at 600 B.U.), %	57.97	58.64	58.08	58.19
Dough-development angle degree	33.72	35.30	44.16	37.71
Weakening area, sq. cm.	3.25	2.78	4.79	3.64
Mean band width, cm.	1.13	1.20	1.19	1.17
DIFFERENCES BETWEEN GRADES				
	F.		5% pt.	
Flour protein, %	118.93		3.18	
Loaf volume, cc.	56.62		3.18	
Absorption (at 600 B.U.), %	0.68		3.18	
Dough-development angle degree	12.74		3.18	
Weakening area, sq. cm.	0.64		3.18	
Mean band width, cm.	4.04		3.18	
STANDARD DEVIATIONS (OF INDIVIDUAL VALUES)				
Flour protein, %	0.34	0.74	0.66	0.78
Loaf volume, cc.	40.27	66.11	88.47	105.20
Absorption (at 600 B.U.), %	1.28	1.88	1.49	1.58
Dough-development angle degree	5.63	7.50	10.61	9.34
Weakening area, sq. cm.	1.41	1.58	3.22	2.39
Mean band width, cm.	0.10	0.14	0.14	0.13
COEFFICIENTS OF VARIABILITY				
Flour protein, %	2.6	5.7	5.5	6.1
Loaf volume, %	4.3	7.5	11.9	12.3
Absorption (at 600 B.U.), %	2.2	3.2	2.6	2.7
Dough-development angle, %	16.7	21.2	24.0	24.8
Weakening area, %	43.4	56.8	67.2	65.7
Mean band width, %	8.6	11.9	11.3	11.1

TABLE II

SIMPLE CORRELATION COEFFICIENTS AND LINEAR REGRESSION EQUATIONS INVOLVING FLOUR PROTEIN, LOAF VOLUME, AND FARINOGRAM MEASUREMENTS—CARGO SERIES

	Absorption	Loaf volume	Dough development angle	Weakening area	Mean band width
SIMPLE CORRELATION COEFFICIENTS <sup>1</sup>					
Flour protein	-.173	+.903	-.735	-.652	+.222
Loaf volume	—	—	-.699	-.619	+.167
Dough-development angle	—	—	—	+.830	-.354
Weakening area	—	—	—	—	-.549
LINEAR REGRESSION EQUATIONS AND STANDARD ERROR OF PREDICTION (cc.)					
Loaf volume = -717.9 + 122.63 flour protein					45.2
Loaf volume = 1149.9 - 7.87 dough-development angle					75.3
Loaf volume = 952.4 - 27.31 weakening area					82.7
Loaf volume = 693.0 + 136.76 mean band width					103.7

<sup>1</sup> Value of  $r$  at 5% point = +.138 (approx.).

The correlation between loaf volume and band width (+.167) is not significant and that between protein content and band width (+.222) only slightly exceeds the 5% level of significance. Mean band width is then neither materially influenced by protein content nor is it related to loaf volume. The high correlation between dough-development angle and weakening area (+.830) would be anticipated from the general form of farinograms for flours of varying strength, since increasing dough-development time is generally associated with decreasing weakening area.

The significantly higher correlation between loaf volume and protein than between loaf volume and dough-development angle or weakening area implies that protein content is the most reliable index of flour strength in this series of samples. This can be illustrated in another way by calculating the standard errors for the prediction of loaf volume from protein content and each of the farinogram measurements. These turn out to be 45 cc. for protein content as compared with 75 and 83 cc. for dough-development angle and weakening area, respectively.

In view of the close similarity between the correlation for protein content and dough-development angle and the correlation for loaf volume and dough-development angle, and the similarity between the corresponding correlations involving weakening area, it appeared that the correlations between these farinograph measures and loaf volume might be largely a reflection of protein content. That this is actually true was shown by calculating the partial correlation coefficients, inde-

pendent of protein content, for loaf volume and each of these farinogram measurements. These are recorded in Table III.

TABLE III  
COMPARISON OF SIMPLE, PARTIAL, AND MULTIPLE CORRELATION COEFFICIENTS—  
CARGO SERIES

SIMPLE VS. PARTIAL CORRELATION COEFFICIENTS			
	Simple	Partial independent of protein	Third farinograph measurement
Loaf volume and dough-development angle	-.699	-.120	—
Loaf volume and weakening area	-.619	-.092	—
Loaf volume and mean band width	+.167	-.084	—
Dough-development angle and weakening area	+.826	+.681	+.813
Dough-development angle and mean band width	-.354	-.241	+.219
Weakening area and mean band width	-.549	-.547	-.489
Value of $r$ at 5% point	+.138 (approx.)	+.138 (approx.)	+.138 (approx.)

SIMPLE VS. MULTIPLE CORRELATIONS		
	Simple	Multiple
Loaf volume and protein	+.903	—
Loaf volume and protein including dough-development angle	—	+.904
Loaf volume and protein including weakening area	—	+.904
Loaf volume and dough-development angle	-.699	—
Loaf volume and dough-development angle, including weakening area	—	+.703
Loaf volume and dough-development angle, including weakening area and mean band width	—	+.705
Value of correlation at 5% point	+.138 (approx.)	+.212 (approx.)

Since the partial correlation coefficient for loaf volume and dough-development angle is not significant ( $-.120$ ), whereas the simple correlation coefficient ( $-.699$ ) is significant, it is apparent that the latter reflects only the correlations between each variable and protein content, and does not represent a real relation between loaf volume and dough-development angle. Similar associations exist between protein content, loaf volume, and weakening area. The partial correlation coefficient for the last two variables, independent of protein content, is not significant ( $-.092$ ), thus showing that no real relation exists between loaf volume and weakening area.

Since the partial correlation coefficients are not significant, it is apparent that the prediction of loaf volume from protein content cannot be improved upon by including farinograph measurements as additional independent variables. This is shown by the comparison of simple and multiple correlations recorded in Table III. The multi-

ple correlation (+.904) between loaf volume, on the one hand, and protein content and dough-development angle on the other hand, is not significantly greater than the simple correlation coefficient for loaf volume and protein content alone (+.903). Similar results are obtained if weakening area rather than dough-development angle is introduced as the second independent variable. It is of interest to note also that the prediction of loaf volume from either dough-development angle or weakening area cannot be improved by including the alternative farinogram measure.

The associations between loaf volume and protein content, loaf volume and dough-development angle, and loaf volume and weakening area, which have just been discussed, are based on the data for all three grades considered as a single series. However, when these relations are studied by means of the scatter diagrams given in Figures 2, 3, and 4, it appears that the No. 3 Northern samples are differentiated from those representing Nos. 1 and 2 Northern as regards the general relations between loaf volume and these three variables. The loaf volume of the No. 3 Northern samples is not as high as would be indicated by the regression equation for loaf volume on protein content, computed for the entire series, and in the instance of dough-development angle and weakening area, the differentiation of No. 3 Northern is even more clearly indicated. This suggests that a protein "quality" factor is entering into the relationships. It would, in fact, be expected that the No. 3 Northern samples would not only be of lower average "gluten quality" but would also exhibit wider variations in gluten characteristics than the samples representing Nos. 1 and 2 Northern, since, in the crop year 1935-36, Garnet wheat was admitted into this grade. It is well known that, among other differences, this variety has gluten characteristics which distinguish it from the varieties of the Marquis type which are eligible for the highest grades. The Garnet content of the No. 3 Northern samples in this series, as determined by growing tests, varied from 0.0% to 64.9% with an average of 30.8%.

It would be expected, therefore, that wider variations in protein "quality" would occur in No. 3 Northern than in Nos. 1 and 2 Northern, and it seemed possible that the farinograph might show up to better advantage, relative to protein content, as an index of loaf volume, in this particular grade. Accordingly, the data for Nos. 1 and 2 Northern combined, and for No. 3 Northern, were analyzed separately; the results of these analyses are recorded in Table IV. The simple correlation between loaf volume and protein content (+.869) for the No. 3 Northern samples is significantly higher than that for loaf volume and dough-development angle (-.738); the correlation between loaf

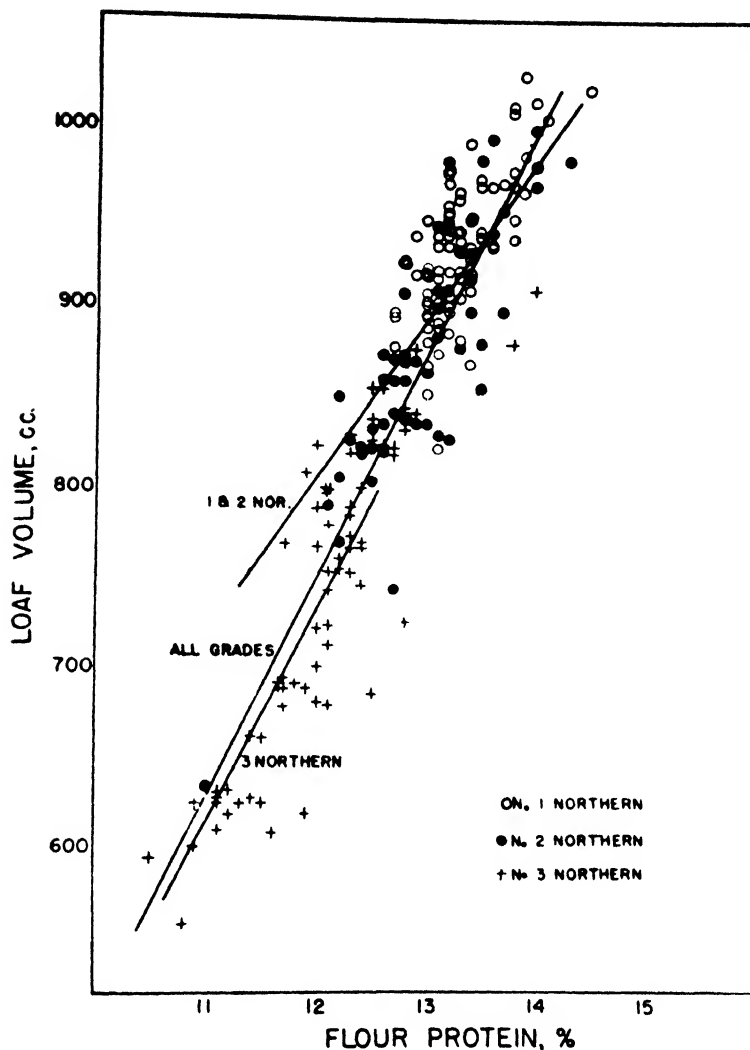


Fig. 2. Scatter diagram for loaf volume and protein content—Cargo series.

volume and weakening area ( $-.769$ ) is not significantly lower than that between loaf volume and protein content although it approaches the 5% level of significance. It can at least be said that the farinograph possesses no advantage over protein content as an index of loaf volume, even in this grade where it would be expected to show up most favourably.

The complicating influence of the relations between the different variables and protein content was again removed by calculating partial correlation coefficients, independent of protein content. Low but significant partial correlation coefficients were obtained for loaf volume and each of the farinograph measures. Accordingly, it might be anticipated that there would be a slight gain in the precision with

which loaf volume could be estimated from protein content by including farinogram measurements as additional independent variables. Comparisons of the multiple correlation coefficients for the combined effects of protein content and dough-development angle on loaf volume ( $+0.879$ ), and the corresponding multiple correlation including

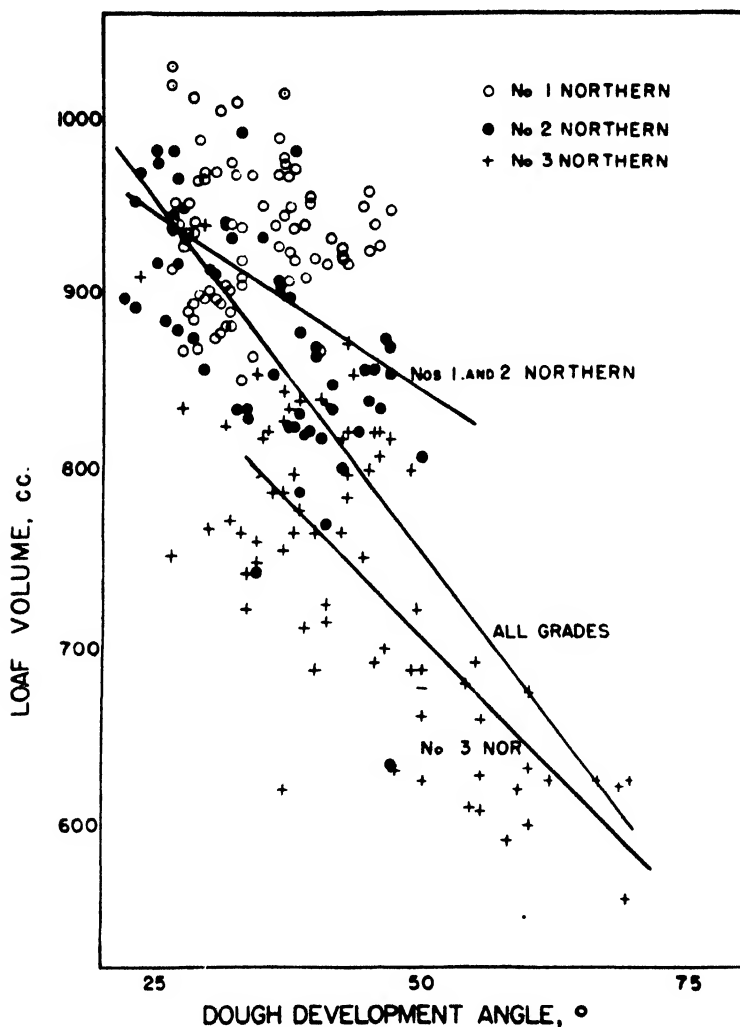


Fig. 3. Scatter diagram for loaf volume and dough-development angle—Cargo series.

weakening area ( $+0.893$ ), with the simple correlation between loaf volume and protein content ( $+0.869$ ), suggest some improvement in prediction. However, an analysis of variance showed that the multiple correlations were not significantly higher than the simple correlations and it can only be concluded that the accuracy of estimating loaf volume from protein content is not increased by including either farinogram measure.

*Other series.*—Similar studies were conducted on the three additional series of hard red spring wheat samples previously mentioned. In these studies measurements were made of both dough-development time, the measurement recommended by Brabender, and of dough-development angle, the corresponding measurement devised in this laboratory. On the other hand, the measurement of band width was

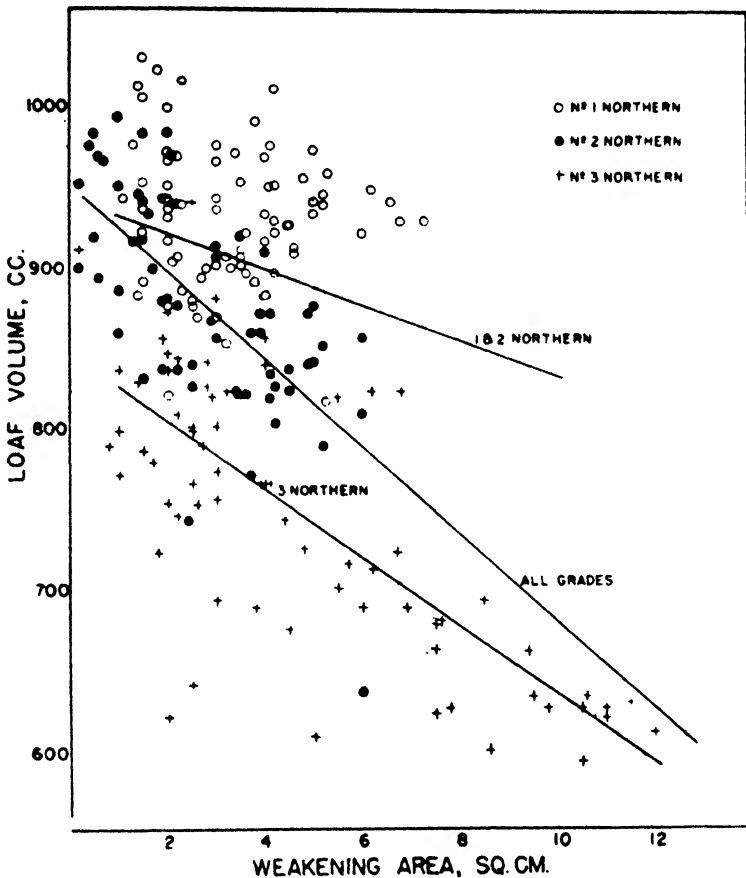


Fig. 4. Scatter diagram for loaf volume and weakening area—Cargo series.

not made, since it was time-consuming and had been proved relatively unimportant in the Cargo series.

The Plant Breeders' variety series consisted of 48 composites, representing certain named varieties and wheat hybrids grown in 1935 in rod rows at various points in the Prairie Provinces. In testing these varieties, all baking tests were conducted in quadruplicate and hence the loaf-volume data utilized in the statistical studies represent the means of four determinations rather than two as in the instance of the other series reported in this paper.



TABLE IV  
STATISTICAL CONSTANTS FOR GRADES NO. 1 AND NO. 2 COMBINED AND  
GRADE NO. 3 NORTHERN, CARGO SERIES

	Mean		Standard deviation	
	No. 1 & No. 2 Northern	No. 3 Northern	No. 1 & No. 2 Northern	No. 3 Northern
Flour protein, %	13.2	12.1	0.52	0.66
Loaf volume, cc.	909.0	744.0	59.27	88.47
Dough-development angle, degrees	34.4	44.2	6.51	10.61
Weakening area, sq. cm.	3.0	4.8	1.52	3.22

LINEAR REGRESSION EQUATIONS AND STANDARD ERRORS OF PREDICTION

No. 1 to No. 2 Northern			cc.
Loaf volume = -232.4 +	86.56	flour protein	38.7
Loaf volume = 1047.1 -	4.015	dough-development angle	55.3
Loaf volume = 942.5 -	10.96	weakening area	56.6
No. 3 Northern			
Loaf volume = -663.1 +	116.48	flour protein	43.8
Loaf volume = 1045.6 -	6.15	dough-development angle	59.7
Loaf volume = 845.2 -	21.13	weakening area	56.6

SIMPLE VS. PARTIAL CORRELATION COEFFICIENTS

	No. 1 and No. 2 Northern			No. 3 Northern		
	Simple	Partial		Simple	Partial	
		Independent of:			Independent of:	
		Protein	Other farino- graph measure		Protein	Other farino- graph measure
Loaf volume $\times$ flour protein	+ .758	—	—	+ .869	—	—
Loaf volume $\times$ dough-development angle	— .358	— .316	— .232	— .738	— .265	— .321
Loaf volume $\times$ weakening area	— .281	+ .156	— .024	— .769	— .414	— .442
Protein $\times$ dough-development angle	— .206	—	—	— .749	—	—
Protein $\times$ weakening area	— .488	—	—	— .722	—	—
Dough-development angle $\times$ weakening area	+ .822	—	—	+ .800	—	—
Approx. value of $r$ at 5% point	—	+ .159	—	—	+ .232	—

SIMPLE VS. MULTIPLE CORRELATION COEFFICIENTS

	No. 1 and No. 2 Northern		No. 3 Northern	
	Simple	Multiple	Simple	Multiple
Loaf volume and protein	+ .758	—	+ .869	—
Loaf volume and protein, including dough-development angle	—	+ .786	—	+ .879
Loaf volume and protein, including weakening area	—	+ .765	—	+ .893
Loaf volume and dough-development angle	-.358	—	-.738	—
Loaf volume and dough-development angle, including weakening area	—	+ .359	—	+ .796
Approx. value of correlation at 5% point	+ .159	+ .244	+ .232	+ .351

The "Winnipeg averages" comprise composites made up of small portions of the wheat of similar grade taken from cars inspected at Winnipeg during the first few weeks of the crop movement. These samples, which represent many hundreds of cars, are collected and tested each year in connection with the annual report of the laboratory on the quality characteristics of the various grades of the new crop. In view of the nature of these samples, it seems worth while to present the individual data for protein content, loaf volume, and farinograph measurements for the crops studied (1936, 1937, and 1938); these are recorded in Table V.

TABLE V  
WINNIPEG AVERAGES OF DIFFERENT GRADES: FLOUR PROTEIN CONTENT,  
LOAF VOLUME AND FARINOGRAPH DATA

Lab. No.	Grade	Absorp- tion at 600 B.U.	Dough development		Weak- ening area	Flour protein	Loaf volume
			Time	Angle			
		%	Min.	Deg.	Sq. cm.	%	cc.
1936 CROP							
2278	1 Hard	57.8	7.6	33.5	4.0	14.3	945
2279	1 Nor.	58.4	7.0	35.0	2.8	14.4	985
2280	2 Nor.	58.8	6.6	38.5	2.8	14.2	993
2281	3 Nor.	57.8	7.0	33.0	2.9	13.6	940
2282	4 Nor.	57.8	7.6	31.5	2.9	13.9	920
2283	1 C. W. Gar.	52.0	6.0	36.0	2.8	11.5	638
2284	2 C. W. Gar.	52.0	6.0	35.0	0	11.5	642
1937 CROP							
4927	1 Hard	58.3	6.5	39.0	3.9	14.1	874
4928	1 Nor.	58.3	5.8	41.5	5.0	13.4	844
4929	2 Nor.	58.0	6.4	36.0	3.6	13.0	795
4930	3 Nor.	57.0	5.8	41.5	3.4	12.9	774
4931	4 Nor.	56.1	5.0	44.5	3.7	12.4	774
4932	No. 5	56.2	5.0	43.0	5.4	12.2	753
4933	4 Special	55.7	4.8	44.0	5.0	11.7	704
4934	5 Special	55.3	4.0	51.0	9.2	11.8	713
4935	1 C. W. Gar.	56.6	5.0	46.0	5.7	12.5	672
4936	2 C. W. Gar.	58.1	6.0	39.5	5.9	13.0	690
1938 CROP							
6094	1 Hard	59.0	5.2	47.0	6.0	13.1	851
6095	1 Nor.	59.1	5.0	44.5	5.2	13.6	866
6096	2 Nor.	58.1	6.5	35.5	2.8	13.2	882
6097	3 Nor.	57.9	5.6	41.0	3.2	13.1	843
6098	4 Nor.	56.7	6.5	34.5	3.7	13.4	867
6099	No. 5	56.7	4.2	49.5	7.6	12.2	725
6100	No. 6	55.9	4.4	48.5	7.2	11.9	742
6102	4 Special	54.1	5.5	39.0	4.5	13.0	804
6103	5 Special	53.9	6.0	36.5	3.5	13.0	813
6104	6 Special	53.5	4.0	49.5	5.3	12.7	818
6105	1 C. W. Gar.	58.1	4.2	50.5	5.9	11.8	598
6106	2 C. W. Gar.	59.3	4.5	46.0	7.0	11.7	612
6107	3 C. W. Gar.	59.1	3.8	51.5	9.2	12.0	626

The frosted wheat series consists of a portion of the 1935 crop samples collected in connection with a study of the quality and grading of frosted wheat (Malloch, Geddes, Larmour, and McCalla, 1937).

The statistical constants for the three series of samples are summarized in Table VI and require little comment. The high correlations between dough-development time and dough-development angle in all three series and the close similarity in the numerical values for the correlations between these respective measures and other variables indicate that the dough-development angle, utilized in the Cargo series, was an adequate and reliable measure of the dough-development time.

Flour absorption is not significantly correlated with dough-development time, weakening area, or loaf volume, but there is a low but significant correlation between this variable and protein content in the Plant Breeders' and Winnipeg Average series. Markley, Bailey, and Harrington (1939) found high and significant correlations between absorption and these variables in a diverse group of 89 flours. These investigations indicated that the relation between absorption and dough-development time was probably nonlinear. Previously, Bailey (1930) emphasized the curvilinear relation between plasticity of doughs and the water used in their formation and mentioned that the logarithm of the plasticity plotted against the percentage of water approached a straight line. In the present study, the authors were primarily concerned with the associations between loaf volume, protein content, and farinograph measures, and the nature of the relations involving absorption has not been studied in detail.

The correlations between dough-development time and weakening area are of similar magnitude in all three series and the degree of association between these two variables and loaf volume does not differ statistically; these relations are in agreement with those noted for the Cargo series.

In all three series protein content is positively correlated with loaf volume, the coefficient for the Plant Breeders' variety series being the lowest. This may be due in part to the relatively high protein content and narrow range between samples and is probably also a reflection of differences in gluten characteristics in such hybrid material.

In the frosted wheat series the spread in protein content is fairly wide and a fairly high correlation coefficient is therefore obtained. It should be noted that in this series the gluten characteristics of the samples are probably very similar since it is well known that "bran frost," the type of damage prevailing in these samples, has little effect on baking strength, as indicated by loaf volume. In the Winnipeg average series, while the lower grades and Garnet grades differ materially in gluten quality from the higher grades, the very wide range in

TABLE VI  
STATISTICAL CONSTANTS FOR PLANT BREEDERS' VARIETIES, WINNIPEG  
AVERAGES, AND FROSTED WHEAT SERIES

Series	Absorption	Dough development		Weakening area	Flour protein	Loaf volume
		Time	Angle			
	%	Min.	Deg.	Sq. cm.	%	Cc.
MEANS						
Plant breeders' varieties	58.37	6.13	40.61	3.84	14.72	921.00
Winnipeg averages	56.85	5.58	41.40	4.67	12.84	790.10
Frosted wheat	60.79	6.00	42.59	4.84	12.26	677.40
STANDARD DEVIATIONS						
Plant breeders' varieties	1.63	1.21	6.63	2.10	0.86	75.93
Winnipeg averages	2.04	1.08	6.05	6.43	0.88	112.50
Frosted wheat	1.94	3.16	17.22	3.80	1.56	125.23
COEFFICIENTS OF VARIABILITY						
Plant breeders' varieties	2.8	19.7	16.3	54.7	5.8	8.2
Winnipeg averages	3.6	19.4	14.6	137.7	6.9	14.2
Frosted wheat	3.2	52.7	40.4	78.5	12.7	18.5
SIMPLE CORRELATION COEFFICIENTS						
Plant breeders' varieties <sup>1</sup>						
Absorption	---	+.213	---	-.227	+.336	+.173
Dough-development time	---	---	-.949	-.781	+.286	+.084
Dough-development angle	---	---	---	+.822	-.131	-.052
Weakening area	---	---	---	---	+.193	-.009
Flour protein	---	---	---	---	---	+.538
Winnipeg averages <sup>2</sup>						
Absorption	---	+.145	---	+.076	+.460	+.319
Dough-development time	---	---	-.952	-.754	+.735	+.690
Dough-development angle	---	---	---	+.826	-.566	-.558
Weakening area	---	---	---	---	-.393	-.445
Flour protein	---	---	---	---	---	+.915
Frosted wheat series <sup>3</sup>						
Absorption	---	-.089	---	+.119	+.147	-.088
Dough-development time	---	---	-.922	-.671	+.827	+.852
Dough-development angle	---	---	---	+.664	-.835	-.853
Weakening area	---	---	---	---	-.723	-.757
Flour protein	---	---	---	---	---	+.871
LINEAR REGRESSION EQUATIONS						
						Error of prediction
Plant breeders' varieties	Loaf volume =	222.5 + 47.45 protein				Cc.
Winnipeg averages	Loaf volume =	-715.3 + 117.24 protein				64.0
Frosted wheat series	Loaf volume =	-179.8 + 69.92 protein				45.4
Winnipeg averages	Loaf volume =	388.9 + 71.88 dough-development time				61.5
Frosted wheat series	Loaf volume =	474.82 + 33.76 dough-development time				81.4
Winnipeg averages	Loaf volume =	826.4 - 7.78 weakening area				65.6
Frosted wheat series	Loaf volume =	798.2 - 24.97 weakening area				100.7
						81.8

## SIMPLE VS. FIRST-ORDER PARTIAL CORRELATION COEFFICIENTS

	Plant breeders' varieties		Winnipeg averages		Frosted wheat series	
	Simple	Partial holding protein constant	Simple	Partial holding protein constant	Simple	Partial holding protein constant
Loaf volume and protein	+.538	---	+.915	---	+.871	---
Loaf volume and dough-development time	+.084	-.087	+.690	+.062	+.852	+.478
Loaf volume and weakening area	-.009	-.137	-.445	-.229	-.757	-.374
Approx. value of correlation at 5% point	+.288	+.288	+.361	+.367	+.304	+.325

<sup>1</sup> At 5% point  $r = +.288$  (approx.).<sup>2</sup> At 5% point  $r = +.361$ .<sup>3</sup> At 5% point  $r = +.304$  (approx.).

TABLE VI—*Continued*

	SIMPLE VS. MULTIPLE CORRELATION COEFFICIENTS					
	Plant breeders' varieties		Winnipeg averages		Frosted wheat series	
	Simple	Multiple	Simple	Multiple	Simple	Multiple
Loaf volume and protein	+ .538	—	+ .915	—	+ .871	—
Loaf volume and protein plus dough-development time	—	+ .543	—	+ .915	—	+ .902
Loaf volume and protein plus weakening area	—	+ .550	—	+ .919	—	+ .890
Loaf volume and dough-development time	+ .084	—	+ .690	—	+ .852	—
Loaf volume and weakening area	— .009	—	— .445	—	— .757	—
Loaf volume and dough-development time plus weakening area	—	+ .122	—	+ .693	—	+ .887
Approx. value of correlation at 5% point	+ .288	+ .430	+ .361	+ .538	+ .304	+ .470

protein content masks the interfering effects of quality differences, and a high correlation coefficient is obtained.

One would anticipate that if the farinograph curve characteristics reflect baking strength, they would prove most closely associated with loaf volume in Plant Breeders' material where protein quality may be expected to vary most widely. It might even be anticipated that the associations would prove to be of a higher degree than that between loaf volume and protein content. In the Plant Breeders' series, however, the correlations between loaf volume and dough-development time (+.084) and between loaf volume and weakening area (— .009) are not significant. Thus expectations are not realized. In the frosted wheat series, on the other hand, the correlations for loaf volume and dough-development time (+.852), and for loaf volume and weakening area (— .757), compare quite favourably with that between loaf volume and protein content (+.871). Corresponding correlation coefficients for the "Winnipeg averages" hold an intermediate position. The correlation for loaf volume and protein content (+.915) is significantly higher than those for loaf volume and dough-development time (+.690) or weakening area (— .445), but the last two coefficients are significant and fairly high. The result is that loaf volume may be more accurately predicted from protein than from either farinograph measure in all series. It should be noted, however, that in the frosted wheat series the correlation between loaf volume and dough-development time is not significantly lower than the correlation between loaf volume and protein content.

The partial correlation coefficients show, as in the Cargo series, that the associations between farinogram measurements and loaf volume reflect mainly the relations between each variable and protein content. When the effects of differences in protein content are removed by calculating partial correlation coefficients, these are found to be insignificant except in the frosted wheat series in which low but significant values

are obtained. However, the multiple correlations again reveal that no significant increase in precision in estimating loaf volume from protein content can be obtained by inclusion of dough-development time or weakening area. Moreover, the estimation of loaf volume from dough-development time or weakening area is not improved by the combination of both farinograph measures.

### Discussion

These statistical studies of the relations between protein content, normal farinogram characteristics, and loaf volume on 333 samples of experimentally milled hard red spring wheat flours, indicate quite clearly that with such material protein content is on the whole a better index of loaf volume, as determined by the routine testing procedure employed in this laboratory, than any of the farinograph measures. The mean band width is only very slightly influenced by variations in protein content and is not significantly correlated with loaf volume. In this connection it is of interest to note that Markley (1937) found that starch-water systems gave quite broad lines in the farinograph and Markley and Bailey (1938) conclude that the band width is essentially a function of the mobility of the dough and is not a measure of its elasticity. While the dough-development time and weakening area are, respectively, positively and negatively correlated with loaf volume, the variations in these measures are largely a reflection of differences in the protein content of the flours and they give, on the whole, lower correlations with loaf volume than that between protein content and loaf volume.

It has been well established that protein content is the major factor affecting the baking capacity of Canadian hard red spring wheat. That variations in gluten quality may diminish this relation has been shown by Malloch, Geddes, Larmour, and McCalla (1937) in extensive investigations of the milling and baking quality of frosted wheat. These investigators found that the correlations between protein content and loaf volume within grades were higher than for all grades combined, indicating that the correlation is affected by the proportion of damage in the sample and that the relation can be improved if this is taken into account.

If the characteristics of the normal farinogram reflect both protein content and protein "quality," it might be expected that the prediction of loaf volume could be made more accurately from dough-development time or weakening area, or from both, than from protein content alone. This is not so, and while there are indications in the present study that the characteristics of the farinograph curve are influenced to some extent by the character of the gluten, the effects on the curve are not

sufficient to render the farinograph of any practical advantage, when combined with protein determinations, in estimating baking strength as reflected by loaf volume.

The question arises as to which of the two farinogram characteristics, dough-development time or weakening area, is more closely related to loaf volume. In all four series of samples the correlations between these measures and loaf volume were not significantly different but it is of interest to note that the numerical values for the correlations involving dough-development time (or angle) were usually the higher. Moreover, in the Cargo series, the mean weakening areas for the three grades were not significantly different and it would appear that, on the whole, dough-development time is the preferable value to use as it is much the more readily determined. Markley and Bailey (1938a) have pointed out that the rate of increase in the mobility of flour doughs upon overmixing is a function of many factors including mechanical degradation of the gluten structure, proteolytic activity, the thixotropic nature of starch in the presence of water, the action of alpha-amylase, the presence of embryo constituents, and the influence of baking ingredients. In these circumstances, it is apparent that weakening area is affected by many factors and it cannot be expected to provide a simple and direct measure of flour strength.

Although baking strength, as reflected by loaf-volume-producing ability, is the most important factor contributing to the baking value of Canadian wheats, it must be borne in mind that, in the commercial evaluation of world wheats, other factors are also taken into account. Among these, dough characteristics are of considerable importance. Moreover, in wheats of the same general type, dough characteristics may vary independently of baking strength. This tendency is frequently exhibited by samples representing different varieties of the same type of wheat grown at the same station. On the other hand, in bulk shipments of commercial grades, which represent composites of similar varieties grown under different environmental conditions, there is little indication of independent variations in baking strength and dough properties.

It is clear that the normal farinogram is less useful than protein content for estimating the baking strength of Western Canadian hard red spring wheat. The question arises, however, as to whether the farinograph provides useful information concerning dough characteristics. The present investigation does not bear directly on this problem, but the authors, after several years' work with the machine and a careful consideration of the literature on it, are forced to conclude that it has not yet been established that the farinograph is of any use in assessing such differences in dough characteristics as may occur in

flours made from the same general type of wheats, as for instance Western Canadian hard red spring wheats. Nevertheless, this possibility cannot yet be wholly discounted.

On the other hand, it appears that without possessing the merits originally claimed for it, the farinograph still retains a position as a useful laboratory tool. It has proved, for example, a useful guide in estimating flour absorption. The instrument also appears to be of value in estimating the optimum mixing time, both in the experimental laboratory and the commercial bakery. Markley, Bailey, and Harrington (1939) have suggested that in the experimental baking test it would be advisable to employ a variable rather than a fixed mixing time, and this seems worthy of careful investigation.

In addition to determining optimum mixing time, the farinograph might also prove useful in determining mixing tolerance, including the tendency of doughs to become sticky. With a little experience, the point where stickiness develops is easily detected from the farinogram, and this property of doughs materially influences the shape of the curve. Indeed, it is quite possible that the relatively poor correlation between weakening area and loaf volume may be due in part to this factor. While the correlations between loaf volume and dough-development time were not significantly higher than those for loaf volume and weakening area, it is of interest to note that the numerical values for the former were higher in all series except for the No. 3 Northern Cargo samples.

### Summary

The 300- and 50-gram farinograph mixers gave similar normal curve characteristics with flours of varying strength, but the replicability of the tests in the large mixer was somewhat better.

Normal farinograph curve characteristics are greatly influenced by the dough-consistency level. Increasing the consistency at which the doughs were mixed shortened the dough-development time (*i.e.*, the mixing time required to produce doughs of maximum consistency) and increased the width of the curve and the rate at which the consistency decreased upon overmixing. The 500-unit level was unsatisfactory for strong flours and the 600-unit level was arbitrarily selected for routine studies.

Statistical studies of the relation between loaf volume, flour protein content, and farinograph curve characteristics (determined in the small mixer) on 333 samples of Western Canadian hard red spring wheat revealed higher correlations between loaf volume and protein content than between loaf volume and quantitative measures of the dough-development time, consistency decrease, and mean band width



during "overmixing." Mean band width was not significantly correlated with loaf volume and gave only a low correlation with protein content for the 215 samples where this measure was employed.

Dough-development time and consistency decrease were highly and negatively correlated. Dough-development time and consistency decrease were, respectively, positively and negatively correlated with protein content and with loaf volume. Partial correlations, independent of protein content, between these farinograph measures and loaf volume were either not significant or of a low order of magnitude, thus revealing that the correlations between loaf volume and the farinogram measurements are largely a reflection of the correlations between protein content and loaf volume, on the one hand, and protein content and farinogram measurements on the other. Low but significant partial correlations, independent of protein content, between loaf volume and the farinograph measures were obtained in the series in which variations in gluten "quality" probably existed. However, such differences as were measured by the farinograph were not reflected by loaf volume, so that the precision with which loaf volume could be estimated from flour protein content could not be improved significantly by the inclusion of the farinograph measures as additional independent variables.

Correlations for dough-development time and loaf volume, and for consistency decrease and loaf volume, were not significantly different, and the prediction of loaf volume could not be improved by including both farinogram measures.

Since the normal farinogram is of less value than protein content for estimating the loaf-volume capacity of Western Canadian hard red spring wheat flours, its utility would appear to be largely that of providing accessory information on such properties as absorption, optimum mixing time and mixing tolerance.

#### Acknowledgments

The authors are indebted to B. Frisell, whose services were loaned by the Brabender Corporation, Duisburg, Germany, for the preliminary experiments on the effect of absorption and dough-consistency level on the curve characteristics. They also gratefully acknowledge the assistance of Nancy Milton in connection with the statistical reduction of the data.

#### Literature Cited

- Aitken, T. R., and Geddes, W. F.  
1934 The behavior of strong flours of widely varying protein content when subjected to normal and severe baking procedures. *Cereal Chem.* **11**: 487-504.
- Bailey, C. H.  
1930 A method for measuring the relative plasticities of doughs and pastes. *J. Rheology* **1**: 429-432.
- Brabender, C. W.  
1934 Six years farinography. *Cereal Chem.* **11**: 586-597.

Geddes, W. F., *Chairman*

1934 Official A. A. C. C. baking test. *Cereal Chem.* **11**: 363-367.

Malloch, J. G., Geddes, W. F., Larmour, R. K., and McCalla, A. G.

1937 The quality and grading of frosted wheat. Annual surveys of the 1930 to 1935 Western Canadian Crops. *Can. J. Research C* **15**: 567-592.

Markley, Max C.

1937 The colloidal behavior of flour doughs. I. The thixotropic nature of starch-water systems. *Cereal Chem.* **14**: 434-436.

— and Bailey, C. H.

1938 The colloidal behavior of flour doughs. II. A study of the effects of varying the flour-water ratio. *Cereal Chem.* **15**: 317-326.

1938a The colloidal behavior of flour doughs. IV. The causes of the increase in mobility of doughs upon prolonged mixing. *Cereal Chem.* **15**: 708-711.

1939 The colloidal behavior of flour doughs. V. Comparison of the increase in mobility of doughs upon either prolonged mixing or fermentation with the effects of varied mixing times upon loaf characteristics. *Cereal Chem.* **16**: 265-271.

— and Harrington, F. L.

1939 The colloidal behaviour of flour doughs. VI. Dough formation from flours of diverse types. *Cereal Chem.* **16**: 271-279.

Munz, Emil, and Brabender, C. W.

1940 Prediction of baking value from measurements of plasticity and extensibility of dough. I. Influence of mixing and molding treatments upon physical dough properties of typical American wheat varieties. *Cereal Chem.* **17**: 78-100.

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## DOUGH IMPROVEMENT STUDIES. II. THE EFFECT OF ADDING OXIDIZED GLUTATHIONE TO WHEAT-FLOUR DOUGHS

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(Received for publication January 16, 1940)

Through the oxidation by bromate of the reduced glutathione (to be referred to as GSH) present in a dough, a certain amount of oxidized glutathione (to be referred to as GSSG) will exist in that dough. The present study was undertaken to see whether this GSSG itself has any effect on dough properties. The reduced glutathione used was obtained from the firm of Hoffmann-La Roche in Basle, Switzerland. The oxidation was made by titration with a  $n/100$  iodine solution.

The extensimeter tests were carried out with the old type of Chopin extensimeter, but with the new kneader, with which a dough membrane can be obtained without touching the dough (Chopin, 1937). Kent-Jones (1939) has described the two kinds of apparatus, giving at the same time an explanation of the meaning of the results obtained. Duplicate extensimeter tests on nonfermenting doughs from 400 wheats carried out by Avard and Ugrimoff (1938) gave an average difference of 6% for the factor  $W$ , a measure of the mechanical work done. According to Scott Blair and Potel (1937),  $W$  depends partly on the tendency of the doughs to allow gas to escape through it before rupture.

They also state that  $P$  is related to viscosity and should be a measure primarily of water-absorbing capacity, whereas  $G$  is dependent on both "spring" and "shortness."

### Experimental

The curves obtained with the extensimeter for one series are given in Figure 1. There are certain difficulties in handling fermenting

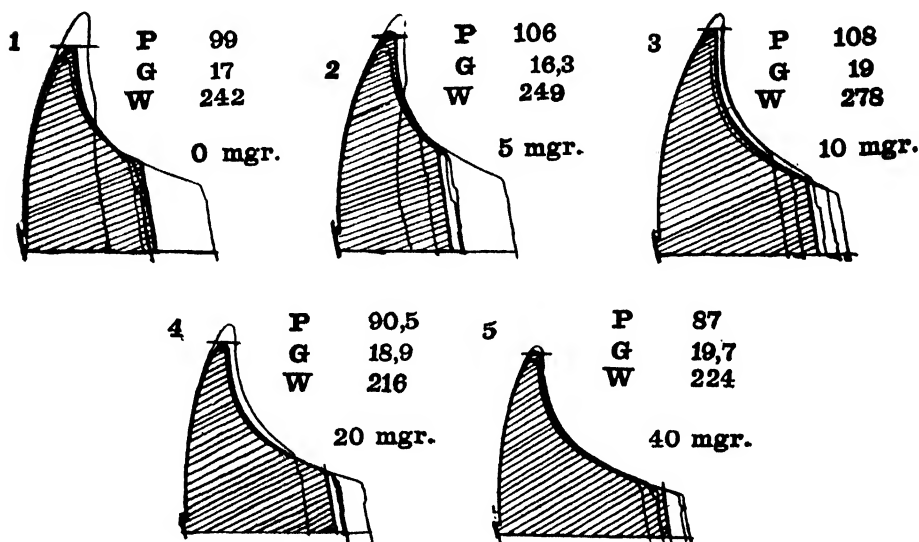


Fig. 1. Extensimeter tests. Doughs made with 250 g. commercial short patent flour, 2% salt, 3% yeast and 50% water. Mixed 8 minutes. Fermentation 25 minutes at 25°C. Amount of GSSG given in mg. per 250 g. flour.

doughs in this test which explain the divergence in the results that arise more from the difficulty of measuring  $G$  than  $P$ . One effect was that with increasing increments of GSSG the dispersion in the height ( $P$ ) and length ( $G$ ) of the five curves decreased. This would be expected if fermentation had been suppressed by the addition, but this was not the case as baking and gassing tests, to be referred to later, showed.

The results of two independent series of extensimeter tests using the same sample of short patent flour are shown in Figure 2, which demonstrates clearly that the tenacity ( $P$ ) of the fermenting dough and the amount of mechanical work needed ( $W$ ) both go through a distinct maximum on the addition of 4 mg. GSSG per 100 g. flour. With larger amounts the dough still retains more spring and is less "short" than the original untreated dough. Bromate, on the contrary, renders dough "short."

Figure 3 brings out the effect, as measured by the farinograph, of the addition of GSSG to a fermenting dough. The arrow indicates

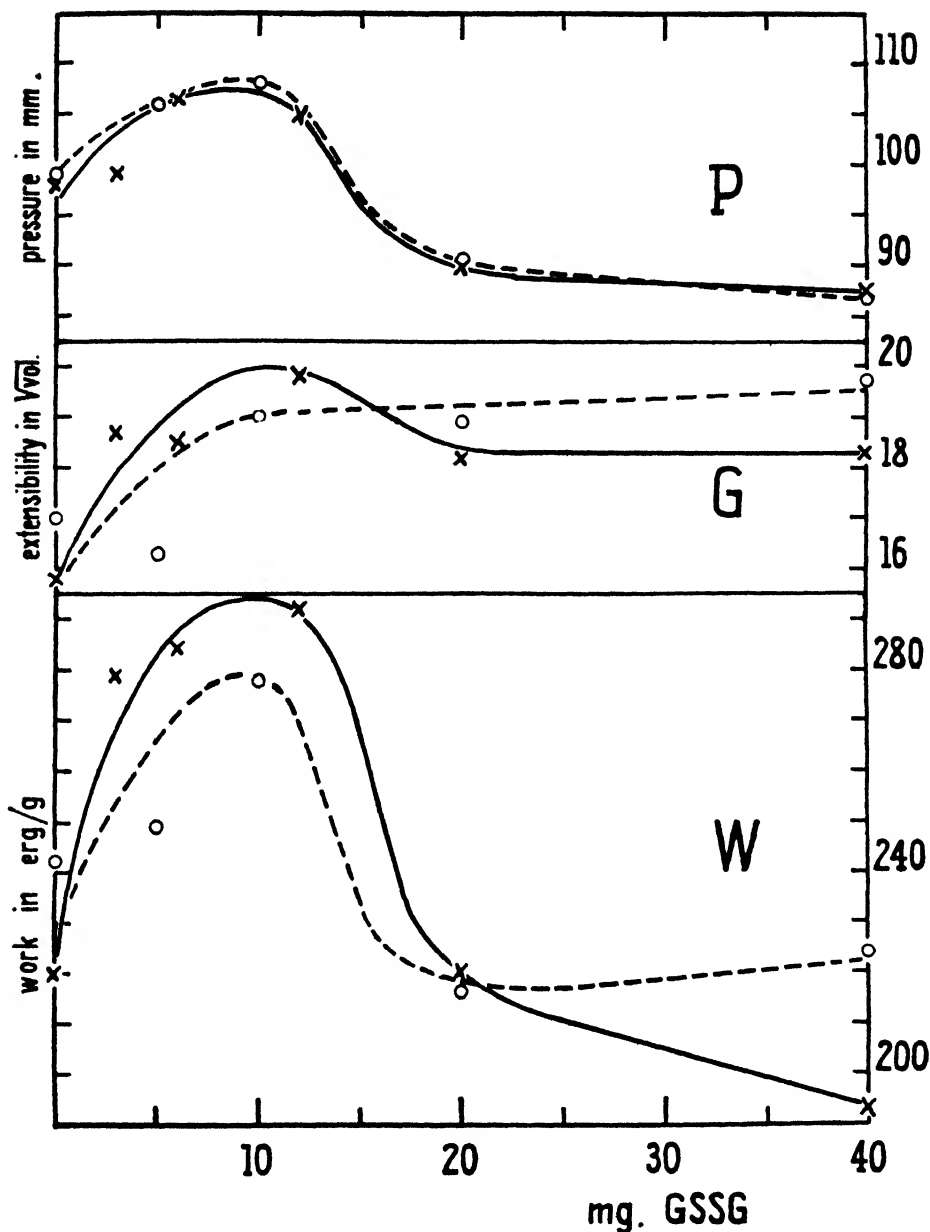


Fig. 2. Results of a duplicate series of extensimeter tests. Doughs as in Figure 1.  
Mg. GSSG given per 250 g. flour.

the point of maximum swelling power, one of the three maxima of the farinogram mentioned by Bungenberg de Jong (1936). With increasing increments of GSSG the swelling time decreases.

The results of baking tests are given in Table I, where characterizations of the doughs are to be found. These results confirm those found with the extensimeter, namely that GSSG itself had an improving

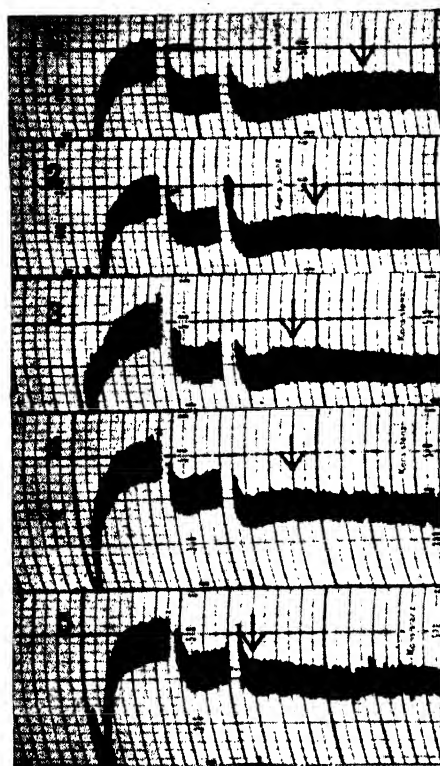


Fig. 3. Farinograms. 300 g. commercial short patent flour, 2% salt, 3% yeast, 56% water. Two rest periods of 60 minutes each. The arrow shows the point of maximum swelling. Amounts of GSSG per 100 g. flour: Curve 1, 0 mg.; 2, 2 mg.; 3, 4 mg.; 4, 8 mg.; 5, 16 mg.

TABLE I

THE EFFECT OF THE ADDITION OF GSH, GSSG,<sup>1</sup> AND BROMATE ON LOAF VOLUME

Supple- ment	Flour	Loaf vol. with supplements (mg.) per 100 g. flour					Max. difference	Remarks on treated doughs
		0	2	5	10	15		
		cc.	cc.	cc.	cc.	cc.		
GSH	I	475	—	430	405	420	-14%	Doughs soft and sticky. Texture very poor.
GSSG	II	515	—	535	525	525	+ 4%	Doughs firm, but lack liveliness. Texture good.
KBrO <sub>3</sub>	II	515	545	—	—	—	+ 6%	Dough lively. Texture good.

<sup>1</sup> Sixty mg. GSH was dissolved in 200 cc. water and oxidized with 19.5 cc. *n*/100 iodine.

effect, which was the most marked for an addition of about 5 mg. GSSG per 100 g. flour. The addition of a larger amount still gives a firm dough, although the latter is not nearly so lively as a bromate-treated dough. The different increments of GSSG did not affect the texture of the finished loaves nor the fermentation time. This was

also confirmed by gassing tests, which showed no difference either in the rate of gas production or in the amount of total gas produced.

Addition per 100 g. flour	Total gas in 5 hours (25 g. flour)
None	264 cc.
2 mg. GSSG	264 cc.
16 mg. GSSG	258 cc.

The oxidation of GSH by iodine is usually explained by the following reaction:  $2\text{GSH} + 2\text{I} = \text{GSSG} + 2\text{HI}$ . The oxidation of 4 mg. GSH will consequently introduce the equivalent of 0.13 cc. *n*/10 HI in a dough. The presence of this amount of acid cannot explain the action attributed to the GSSG. Bailey and Le Vesconte (1924) showed that on increasing the hydrogen-ion concentration (below pH 6.1) the extensibility (*G*) decreases. On adding oxidized glutathione, however, we have always measured an increase in the factor *G* (Fig. 2).

### Summary

It has been shown that oxidized glutathione influences the swelling power of gluten, but has no action on the gassing power of a dough.

A mechanical dough tester, as well as the baking test, revealed that very small amounts of oxidized glutathione (about 1 part in 25,000) had a beneficial effect on a dough. The improvement of baking strength caused by the addition of bromate is not merely due to the suppression of the harmful effect of the protease activator glutathione, but can partly be explained by the subsequent presence of oxidized glutathione in the dough.

### Acknowledgment

The author is indebted to Mr. F. Brassel for assistance with the experimental work.

### Literature Cited

- Avard, P., and Ugrimoff, A.  
 1938 Essais extensimétriques réalisés en double sur blés. Etude statistique. Bull. école franç. meunerie No. 65, p. 11.
- Bailey, C. H., and Le Vesconte, A. M.  
 1924 Physical tests of flour quality with the Chopin extensimeter. Cereal Chem. 1: 38-63.
- Bungenberg de Jong, H. L.  
 1936 Wesen und Bedeutung der Farinographie in kolloidchemischem Sinne. Mühlenlab. 6: 23-28.
- Chopin, M.  
 1937 Nouveaux appareils pour la préparation et l'essai d'extension des pâtes. Bull. école franç. meunerie No. 60, p. 172.
- Kent-Jones, D. W.  
 1939 Modern cereal chemistry (3rd ed.), Northern Publishing Co., Liverpool.
- Scott Blair, G. W., and Potel, P.  
 1937 A preliminary study of the physical significance of certain properties measured by the Chopin extensimeter for testing flour doughs. Cereal Chem. 14: 257-262.

# DOUGH IMPROVEMENT STUDIES. III. FURTHER STUDIES ON THE OXIDATION OF GLUTATHIONE BY BRO- MATE, CHLORATE, AND PERSULPHATE

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(Received for publication January 16, 1940)

Waldschmidt-Leitz and Balls (1935) state that the proteases of seeds are mixtures of an aminopolypeptidase, a dipeptidase and a proteinase; the latter can be activated by sulfhydryl compounds (e.g. cysteine, glutathione). Balls and Hale (1936) express the opinion that wheat flour proteinases are of the papain type. This idea is also shared by Jørgensen (1939). On the other hand Harris (1939) found that flour proteases do not react like papain, and Read and Haas (1939) that wheat malt proteases do not react like papain.

According to the theory of Jørgensen (1935), in support of which he has published further proof (1938, 1939), oxidizing agents used in baking only suppress the activating action of glutathione on the powerful but normally inactive proteolytic enzymes present in wheat-flour doughs. Sullivan, Howe, and Schmalz (1936) caution against too narrow a view regarding the activation of proteases by sulfhydryl compounds, and Bungenberg de Jong (1939) takes the same attitude regarding the bromate action. Freilich and Frey (1939) have discussed the effect of "excess bromate" in a dough. They state that it is evident from some of their work that the effects of bromate and other oxidants are not confined simply to the inhibition of proteases.

By analogy with other biological oxidations it is conceivable that in the natural oxidation process in wheat flour on "aging," several intermediary steps are involved. In the case of respiration the admirable investigations of Szent-Györgyi (1937) and coworkers have actually fixed the numerous steps in oxidation showing exactly the fate of the hydrogen and of the oxygen. The oxidation proceeds by gradual steps because otherwise much too much energy would be lost. This might well be the case in the natural "aging" of flour, in which process the oxidation of glutathione and the inactivation of protease are probably two of the steps.

Comparing the difference between the action of bromate on the one side and oxygen or inorganic peroxides on the other, Baker and Mize (1937) state that, assuming that the reactions produced by oxidizing agents are slow, this difference can be explained by the ability of bromate to remain active in the dough throughout the entire period of mixing and subsequent fermentation, whereas the other reagents—

oxygen and peroxide—are removed either by aeration of the oxygen from the dough by the escaping carbon dioxide, or the decomposition of the peroxide by catalase into oxygen and its subsequent removal by aeration. It has recently been shown that pure glutathione is indeed only slowly oxidized by one of the most potent improvers used in baking, namely potassium bromate (Ziegler, 1940). The pH did not have any marked effect below pH 6. The same applies to the oxidation of GSH by iodate, periodate, and dehydroascorbic acid, as will be shown later.

### Effect of GSH, Alone and in the Presence of Salt

Through the addition of reduced glutathione serious deterioration of gluten suddenly set in after 5 minutes of kneading (Fig. 1, curve

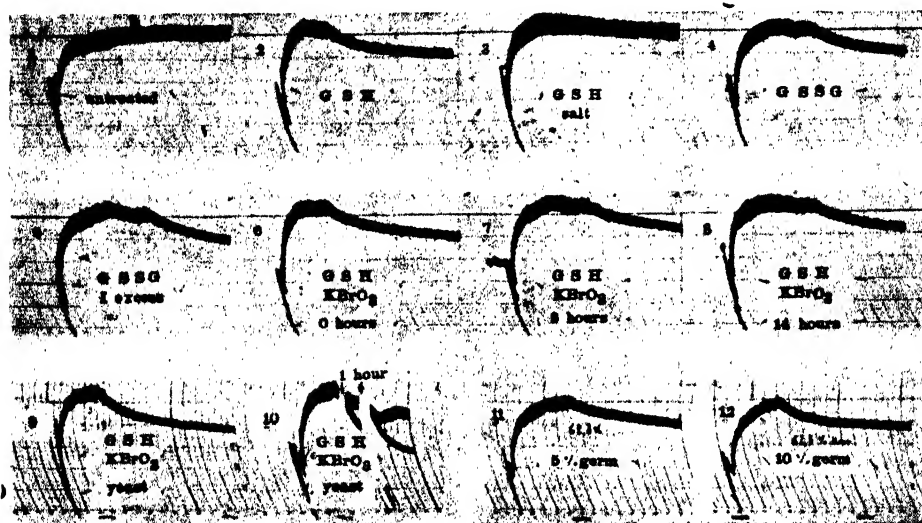


Fig. 1. Farinograms of doughs prepared with 300 g. flour and 180 g. water, without yeast or salt, except where otherwise mentioned. (1) Commercial fresh short patent flour alone. (2) 30 mg. GSH. (3) 30 mg. GSH + 6 g. NaCl. (4) Before addition to dough, 30 mg. GSH oxidized with iodine. (5) Like 4, but with an excess of iodine. (6-8) 30 mg. GSH + 15 mg. bromate allowed to react for 0, 3 and 14 hours. (9) 30 mg. GSH + 15 mg. bromate + 9 g. baker's yeast. (10) Like 9, but with 2 one hour fermentation periods. The curves of the dough without GSH or  $\text{KBrO}_3$  have been drawn in. (11) 95% flour + 5% germ. (12) 90% flour + 10% germ.

No. 2). All authors agree that GSH acts with surprising rapidity in a dough. As Freilich and Frey (1939) have emphasized, papain however only reacts slowly. Probably a specific GSH effect, as well as the enzyme activation effect, takes place in dough.

Sodium chloride is known to toughen or coagulate the gluten (Fig. 1, Nos. 2, 3), so much so that Bungenberg de Jong (1933) maintains that the physical properties of dough should be tested in the absence of salt, which otherwise eliminates the characteristic differences between flours. Curve 3 shows that salt in the proportion used in baking



protected the gluten to a certain extent against the action of added GSH. Table I gives results obtained with the Chopin extensimeter.

TABLE I  
EFFECT OF SALT ON DOUGHS CONTAINING REDUCED GLUTATHIONE,  
MEASURED BY THE CHOPIN EXTENSIMETER.  
250 g. flour. 130 cc. water. P = tenacity; G = extensibility; W = mechanical work.

Short patent flour	Extensimeter factor	No NaCl	5 g. NaCl
No GSH	P	71	75
	G	18.8	21.4
	W	144	214
25 mg. GSH	P	48.5	53.5
	G	15.3	21.4
	W	61	109

The addition of 0.01% GSH considerably reduced the water-absorbing capacity, as measured by the factor *P*, and at the same time made the dough very short. The addition of 2% of salt tightened up the doughs without and with GSH. From these extensimeter curves we see that 2% salt did not completely protect the gluten from the action of 0.01% GSH.

The percentages of salt and glutathione added to the extensimeter and to the farinograph doughs were the same. Both farinograph curves (Fig. 1, Nos. 1, 3) needed the same amount of water (180 cc.) to reach the consistency line 500. For the extensimeter curves in each case 130 cc. of water was added with the result that the doughs containing GSH were softer. Consequently the heights of the farinogram and extensimeter curves are not measures of the same factor. The claim is made for both machines that the water absorption is measured. The following figures show the results:

	Without NaCl	With NaCl	Dough, due to NaCl
(a) Without GSH			
Farinograph consistency "500" (cc.)	180	174	Softer
Extensimeter <i>P</i> (mm.)	71	75	Firmer
(b) With GSH			
Farinograph consistency "500" (cc.)	180	180	Same
Extensimeter <i>P</i> (mm.)	71	53.5	Much softer

### Oxidation of GSH by Chlorate

Over 20 years ago, when Kohman and his assistants announced one of the most important discoveries in cereal chemistry, namely the improving effect of bromate and iodate, they had noticed that chlorate had no action in a dough. Jørgensen (1935) used this fact as proof for his theory. However at one time sodium chlorate was being used in the baking industry and has been recommended as an oxidizing agent

by Working (1928). It can be shown that the high mobility of a flour-water dough containing some papain and allowed to stand for one hour can be reduced by the presence of bromate, but not of chlorate.

Hoffmann (1912) and Hoffman, Ehrhart, and Schneider (1913) tested the activation of chlorate by osmium tetroxide, a very strong oxidizing agent, whose reduction product, osmium hydrate  $\text{Os}(\text{OH})_4$ , is black. For this reason osmium tetroxide is often resorted to in microscopical work for the detection of unsaturated fatty acids. The aleurone cells and germ are colored black by  $\text{OsO}_4$ . Hoffmann states that chlorate alone, in neutral or slightly acid solution, is not an oxidizing agent at all. He also mentioned that  $\text{OsO}_4$  is an oxygen carrier. In the presence of chlorate a compound chlorate— $\text{OsO}_4$  is formed and this acts as the oxidant, the chlorate then giving off all its oxygen.

Tests were undertaken to see whether, in the presence of  $\text{OsO}_4$ , chlorate can oxidize glutathione. The same micro-titrimetric method was used as in a former investigation. A 2% solution of  $\text{OsO}_4$  as used in microscopy was taken, but it should be mentioned that this solution was no longer quite fresh. The figures in Table II show the results.

TABLE II

## OXIDATION OF GLUTATHIONE (GSH) BY CHLORATE

In all cases 1 mg. GSH in 10 cc. distilled water. Room temperature

No.	Substance added	Reaction time		$n/500$ iodine	Degree of oxidation (approx.)
		mg.	min.		
1	None	—	—	1.70	—
2	Potassium bromate	5	0	1.65	0
3	Potassium chlorate	5	0	1.70	0
4	Osmium tetroxide	1.5	0	1.40	18
5	Potassium chlorate + osmium tetroxide	5 1.5	0	0.85	50
6	Potassium bromate	5	60	0.65	60
7	Potassium chlorate	5	60	1.70	0
8	Osmium tetroxide	1.5	60	0.80	50
9	Potassium chlorate + osmium tetroxide	5 1.5	60	0.25	100

Whereas 5 mg. bromate oxidized 1 mg. GSH to the extent of 60% in one hour (Ziegler, 1940), chlorate alone had no action (Table II, No. 6, 7), confirming the well-known fact.  $\text{OsO}_4$  alone (No. 8) oxidized GSH about as quickly as bromate did. Chlorate, in the presence of  $\text{OsO}_4$ , oxidized GSH twice as rapidly as  $\text{OsO}_4$  alone and twice as rapidly as the same amount of bromate. The rate of oxidation of GSH was greater by the combination chlorate- $\text{OsO}_4$  than by any one of the two compounds alone. On baking (hand-mixing) the expected improvement did not occur, as the figures in Table III show.

TABLE III  
EFFECT OF THE ADDITION OF CHLORATE AND OSMIUM OXIDE  
Fermentation time 90 minutes. Experimental flours from Plate wheats.

Additions in mg. per 100 g. flour					
Chlorate	—	2	2	4	—
OsO <sub>4</sub>	—	3	4	3	—
Bromate	—	—	—	—	2
Loaf volume in cc. per 150 g. dough					
Flour I	480	—	490	—	—
Flour II	470	465	—	455	515

The fermentation rate seemed to be unaffected by the addition, and the dough was somewhat stickier than the untreated dough. It is thought that the OsO<sub>4</sub> is reduced by the unsaturated fatty acids and is consequently not free to act in combination with the chlorate.

#### Oxidation of GSH by Bromate

A ratio for GSH: KBrO<sub>3</sub> = 2 : 1 was chosen because, in a former study, GSH was shown to be only slowly oxidized under this condition, and we wanted to test out whether the rate of oxidation would be increased by the presence of dough constituents (Fig. 1, No. 2, 6-8). The GSH and bromate solutions were allowed to react together for 0, 3, and 14 hours before being used as part of the absorption water in a mixing test. According to previous results roughly 0, 25, and 75% of the GSH could be expected to be oxidized by the amount of bromate used in 0, 3, and 14 hours respectively. The same phenomenon appears as when the oxidation is done by iodine (curve No. 4). After a certain time a definite kind of gluten breakdown occurred. As in our titration tests already published, a progressive oxidation of GSH by bromate took place and it did not appear to be catalyzed by the constituents of a nonfermenting dough.

Curves 6 and 9 (Fig. 1) show that the presence of yeast does not increase the rate of oxidation. Two fermenting doughs without salt were made (curve No. 10), with and without a mixture of GSH and bromate. Another test made with GSH and yeast without fermentation period, gave much the same curve as No. 2. Even after two hours (curve No. 10) the bromate did not stop further injury to the gluten, any more than in a nonfermenting dough, again showing that flour constituents do not appear to increase the rate of oxidation of glutathione by bromate.

Although curves No. 11 and No. 12 were prepared some 4 years ago, they are given here as they show the same type of gluten breakdown, curves No. 8 and No. 11 as well as No. 2 and 12 being very similar.

Sullivan, Howe, and Schmalz (1937) have shown that by heating germ or glutathione under increased pressure the deleterious effect can be inhibited. In view of the work of Harington and Mead (1935) who have shown that glutathione can be easily destroyed by boiling in aqueous solution, yielding glutaminic acid and cystylglycyl anhydride, it is possible that the boiling under increased pressure (which results in a boiling point of water above 100°C.) caused a destruction of the molecule rather than an ordinary oxidation to oxidized glutathione.

### Oxidation of GSH by Iodine

Iodine, known to oxidize GSH immediately, was used in a former study to follow the rate of oxidation by bromate (Fig. 1, Nos. 2, 4, 5). Thirty mg. GSH was oxidized with iodine just before the farinograph test was made (curve No. 4). Comparing Nos. 2 and 4, it will be seen that in the latter dough the same type of gluten breakdown as in the GSH dough (No. 2) and in the bromate tests (Nos. 6-8) occurs. With completely oxidized glutathione (No. 4) the breakdown occurred 2 minutes later than in the GSH dough. The oxidized glutathione seems to be reduced again in the nonfermenting dough.<sup>1</sup>

### Oxidation of GSH by Persulphate

As Kent-Jones (1939) points out, Plate and English wheat flours are especially responsive to persulphates, which have been in use for many years in Europe. Baking tests show that about three times as much persulphate as bromate is needed to improve flour and that the action is different in each case. According to Read and Haas (1937) Elion and Elion patented a process in 1932 claiming that persulphates increase the saccharogenic activity of flour very appreciably. Flohil (1936) was able to inhibit the injurious effect of malted flour extract on gluten by ammonium persulphate, among other oxidizing agents. Kosmin (1934) showed that this salt can even damage gluten.

Titration tests were carried out to see to what extent persulphate was able to oxidize pure glutathione. Figures in Table IV show that an amount (50 mg.) which would be amply sufficient for oxidizing 1 mg. GSH in 60 minutes by bromate had hardly any action. The following figures taken from Tables II and IV show that it needs more than 30 times as much persulphate to oxidize GSH as fast as bromate does.

One mg. GSH treated with:	Degree of oxidation after 60 minutes
5 mg. bromate	60%
50 mg. persulphate	15%
150 mg. persulphate	40%

<sup>1</sup> Skovholt and Bailey (Cereal Chem. 12: 321-355, 1935) emphasize the rapid increase in reducing sugars during mixing. GSSC can be reduced by maltose.

TABLE IV

EFFECT OF SOME SUBSTANCES ON THE REDUCING POWER OF GLUTATHIONE  
In all cases 1 mg. GSH in 10 cc. distilled water. Room temperature.

No.	Substance added	Reaction time		n/500 iodine	Degree of oxidation (approx.)
		mg.	min.		%
1	Potassium persulphate	50	60	1.45	15
2	Potassium persulphate	150	60	1.00	40
3	Potassium thiocyanate •(rhodanide)	5	60	1.75	0
4	Formaldehyde (38%)	2 drops	0	1.70 <sup>1</sup>	0
5	Formaldehyde	2 drops	60	1.65	0

<sup>1</sup> Two drops formaldehyde alone needed 0.1 cc. iodine.

Therefore it would seem that although bromate action in baking can be explained to a certain extent by its oxidation of GSH, the improvement by persulphate is mainly due to action on other factors.

#### Effect of Thiocyanate on GSH

In a study on flour improver action Flohil (1936) reported baking tests which showed that KCNS added in fair quantities is of real value as an improver, although its toxic character makes it absolutely unfit for human consumption. As KCNS also inhibited proteolytic action in his tests it was considered worthwhile to see what effect it might have on glutathione. There was no action on the reducing power of GSH by a small amount of KCNS (Table IV). The improvement is due to the big increase it causes in the swelling power of gluten, according to Bungenberg de Jong (1936), and not to any effect on glutathione.

#### Effect of Formaldehyde in Dough

In view of the work of Fujita and Numata (1939), who have recently mentioned that formaldehyde can partially destroy the glutathione molecule, titrations were made (Table IV, Nos. 4, 5). It could not be expected that it would oxidize GSH, being a reducing agent itself. No effect was noticed in the titration. Formaldehyde has a powerful action on the coagulation of gluten (Resnitschenko and Popzowa, 1934; Kosmin, 1934).

Although not bearing directly on the problem treated in this paper, it may be interesting to mention the effect of formaldehyde on the gassing power of dough. Here is a case where reliance on a maltose figure alone leads to very wrong conclusions. In the presence of formaldehyde—as was observed in the author's laboratory—the production of reducing sugars is almost normal, as measured by the

usual diastatic activity determinations. In spite of this fact no gas is produced, showing that only the last stages of alcoholic fermentation are affected by the presence of the aldehyde. It could be shown that neither maltose nor glucose are fermented by yeast in the presence of formaldehyde, known as a strong enzyme poison.

### Summary

Sodium chloride only partially protected gluten against the direct or indirect action of reduced glutathione.

Chlorate is known to be useless as a bread improver and to exert no action on the oxidation of glutathione by itself. The compound which is formed between chlorate and osmium tetroxide oxidized glutathione more rapidly than osmium tetroxide alone and more rapidly than the same amount of bromate. In a dough, however, no improvement was noticed. This may be due to a reaction of the unsaturated fatty acids of flour with the osmium oxide.

The slow oxidation of glutathione by bromate was followed in a nonfermenting and in a fermenting dough.

Glutathione oxidized by iodine or bromate recovered its deleterious effect in a dough containing neither salt nor yeast after several minutes of mixing, probably due to the production of reducing sugars.

It has been shown that the action of the widely used bread improver persulphate must be mainly due to factors other than the oxidation of glutathione, which does proceed although extremely slowly.

The improvement by the addition of thiocyanate is not connected with any action on glutathione. It is known to have a powerful swelling effect on gluten.

The height of the farinogram and of the Chopin extensimeter curve did not measure the same factor, although both are said to be a measure of water absorption.

### Acknowledgment

The author wishes to express appreciation for the valuable assistance of Mr. F. Brassel with the experimental part.

### Literature Cited

- Baker, J. C., and Mize, M. D.  
1937 Mixing doughs in vacuum and in the presence of various gases. *Cereal Chem.* **15**: 201-205.
- Balls, A. K., and Hale, W. S.  
1936 Proteolytic enzymes of flour. *Cereal Chem.* **13**: 54-60.
- Bungenberg de Jong, H. L.  
1933 Die Bedeutung der Mehl-Wasser-Farinograph-Kurve. *Z. ges. Getreide- u. Mühlenw.* **20**: 118-120.  
1936 Wesen und Bedeutung der Farinographie in kolloidchemischem Sinne. *Mühlenlab.* **6**: 23-28.  
1939 Proteins in the flour industry. Symposium on Proteins, Amsterdam, 1938, pp. 160-180.

- Flohil, J. T.  
1936 The effect of chemical flour improvers on proteolytic action. *Cereal Chem.* **13**: 675-689.
- Freilich, J., and Frey, C. N.  
1939 Dough oxidation and mixing studies, III. *Cereal Chem.* **16**: 503-512.
- Fujita, A., and Numata, I.  
1939 Über die kolorimetrische Bestimmung des Glutathions. *Biochem. Z.* **299**: 249-273.
- Harington, C. R., and Mead, T. H.  
1935 Synthesis of glutathione. *Biochem. J.* **29**: 1602-1611.
- Harris, R. H.  
1939 A study of gluten protein fractionation from sodium salicylate solution. III. The effect of proteolytic enzymes. *Cereal Chem.* **16**: 78-88.
- Hoffmann, K. A.  
1912 Sauerstoff-Übertragung durch Osmiumtetroxyd und Aktivierung von Chloratlösung. *Ber.* **45**: 3329.
- , Ehrhart, O., and Schneider, O.  
1913 Aktivierung von Chloratlösungen durch Osmium, II. *Ber.* **46**: 1657.
- Jørgensen, H.  
1935 Über die Natur der Einwirkung von Kaliumbromat und analogen Stoffen auf die Backfähigkeit des Weizenmehles. *Biochem. Z.* **280**: 1-37.  
1938 Entwicklung der Theorie der Bromatwirkung 1935-1938. *Mühlenlab.* **8**: 201-208.  
1939 Weitere Studien über die Natur der Bromatwirkung. *Mühlenlab.* **9**: 109-112.
- Kent-Jones, D. W.  
1939 *Modern Cereal Chemistry* (3rd ed.). Northern Publishing Co., Liverpool.
- Kosmin, N.  
1934 Kleberqualität und einige Faktoren, die diese beeinflussen. *Mühlenlab.* **4**: 109-116.
- Read, J. W., and Haas, L. W.  
1937 Studies on the baking quality of flour as affected by certain enzyme actions. *Cereal Chem.* **14**: 752-766.  
1939 Further studies relating to the activation and inhibition of flour proteinase. *Cereal Chem.* **16**: 60-70.
- Resnitschenko, M. S., and Popzowa, A. I.  
1934 Über die Wirkung ungesättigter Fettsäuren auf die kolloidale Beschaffenheit des Klebers. *Mühlenlab.* **4**: 57-62.
- Sullivan, B., Howe, M., and Schmalz, F. D.  
1936 On the presence of glutathione in wheat germ. *Cereal Chem.* **13**: 665-669.  
1937 The effect of heat treatment on wheat germ. *Cereal Chem.* **14**: 489-494.
- Szent-Györgyi, A. v.  
1937 Studies on biological oxidation and some of its catalysts (text in English). Eggenbergersche Buchhandlung, Budapest.
- Waldschmidt-Leitz, E., and Balls, A. K.  
1935 Proteolytic enzymes of seeds. *Handbuch der Lebensmittelchemie* **2**: 773.
- Working, E. B.  
1928 The action of phosphatides in bread dough. *Cereal Chem.* **5**: 223-234.
- Ziegler, E.  
1940 Dough improvement studies. I. Oxidation of glutathione by potassium bromate. *Cereal Chem.* **17**: 460-468.

# RUSSIAN EXPORT WHEATS—COMPOSITION AND CHARACTER OF THE 1938 CROP

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(Received for publication April 27, 1940)

Russia has always been one of the larger wheat-producing countries of the world. From 1909 to 1913 Russia accounted for 19.5% of the world total production, and in the period 1928 to 1932 she produced 17.1% of the total. The exports of Russian wheats for these years were as follows:

TABLE I  
RUSSIAN WHEAT EXPORTS, 1910-13 AND 1930-36

Year	Bushels: 000 omitted	Year	Bushels: 000 omitted
1910	224,986	1932	7,764
1911	142,806	1933	27,435
1912	96,689	1934	20,199
1913	122,063	1935	91,633
1930	1,312	1936	92,800
1931	26,369	—	—

Holland imported from Russia the following quantities of wheat in recent years:

TABLE II  
HOLLAND IMPORTATIONS FROM RUSSIA, 1930-39

Year	Bushels: 000 omitted	Year	Bushels: 000 omitted
1930	4,620	1935	904
1931	11,566	1936	808
1932	3,768	1937	1,480
1933	2,834	1938	4,540
1934	661	1939	1,592

Of the 1938 Russian crop the Netherlands again had a share, and 5,498,000 bushels were imported. The experiences obtained with this crop when technically viewed are very interesting. As to the part of the country where the wheat was grown little is known, but the wheat was shipped from South Russian ports and was probably spring wheat. The various lots were denoted by number, but it was impossible to draw any conclusion from such numbers which might indicate some particular quality or grade. For example in 1937 No. 441/0 was a wheat yielding flour of good baking value, whereas in 1938 a wheat of that number was quite unsuitable for baking purposes.

In this country during the last few years it has been compulsory to



use a relatively high percentage of home-grown wheat in the mill mixture. This has been as high as 35%, millers being at liberty to use for the remaining 65% any wheats available, and these have consisted of wheats from Canada, United States of America (hard winter wheats), Argentina, Russia, and Rumania.

As the numbers under which Russian wheats were sold bore no relation to their respective qualities, it was necessary, in order to avoid trouble, to examine every parcel. The following summarized results of analytical tests show how necessary it was to carry out proper tests before working these Russian wheats into the mixture. Incidentally, it is of interest to record that all the shipments referred to were essentially red or brown-red varieties, vitreous and fairly hard, and included no soft white wheats. The shipments were all, on the whole, fairly clean; any admixture of weed seeds etc. was not more than 0.5%.

In the table which follows the gluten-content figures, wet and dry, refer to the flour.

TABLE III  
CHARACTERISTICS OF RUSSIAN EXPORT WHEAT

Wheat No.	Test weight	Weight per 1000 kernels	Moisture content	Protein (on dry substance)	Gluten	
					Wet	Dry
	<i>Lbs. per bu.</i>	<i>g.</i>	<i>%</i>	<i>%</i>	<i>%</i>	<i>%</i>
*127 R	64.00	29.5	11.5	12.26	31.0	9.4
*139	64.00	29	11.5	12.46	31.0	9.8
†402/3	63.28	30	11.0	13.49	30.6	9.5
*402/4	63.60	27	11.0	13.48	30	9.5
†402/5	63.12	28.5	11.0	12.07	22.6	7.0
*421/2	65.54	31	10.5	13.55	33.9	10.1
*421/3	65.08	31	12.6	13.98	33.2	10.5
*421/32	64.00	32	12.8	—	37.2	11.3
*421/61	67.68	32.5	9.4	13.78	34.2	10.5
†431/3	64.88	24	11.1	12.95	29.5	8.9
*431/4	65.80	23.5	11.1	12.47	28.4	8.7
*431/61	66.24	24.5	10.6	14.03	34.6	11.8
†441/0	62.68	—	10.8	12.95	Not washable	
*441/2	65.16	33.0	10.4	—	31.3	9.5
*441/4	64.44	26.7	11.4	11.4	32.0	10.1
†441/5	63.28	—	10.9	—	36.3	10.5
†441/6	62.88	31.0	10.0	13.9	Not washable	
†441/53	64.00	29.0	12.1	11.44	22.1	7.1

\* Slightly affected by the wheat bug.

† Heavily affected.

‡ Very heavily affected.

### Damage to the Grain due to Wheat Bug Infection

Almost every wheat sample showed damage caused by the wheat bug. The degree of damage ranged from only lightly affected to badly damaged, in fact samples No. 441/0 and No. 441/6 of the 1938 crop were so heavily infected that it was impossible to bake a good loaf

from the flours of these samples; furthermore it was not possible to retain any of the gluten in a washing test (see Table III). Other lots badly infected were No. 402/3, No. 402/5, No. 431/3, No. 441/5 and No. 441/53.

Wheat bug infection was described for the first time by Berliner.<sup>1</sup> Prior to this other investigators had noted and recorded the occurrence of exceptionally bad gluten in some wheats, but were not aware that this insect was responsible for the damage.

The wheat bug makes its attacks by forcing an entry with its proboscis into the berry when it is in the milk stage or when the grain is green, and by this means it extracts the contents of the berry. Apparently at the same time it deposits a matter very rich in proteolytic enzymes. On ripe kernels the damage by the wheat bug is indicated by a pale yellow speck or mark, with a small dark point, and the kernels are often shrivelled. The small spot indicates the place where the bug has pierced the berry.

### Method of Test Milling the Wheat Samples

After the usual preliminary preparation and cleaning, all samples were ground on an experimental mill to produce a straight-run flour of about 15% moisture and 0.54%–0.58% ash, the latter calculated on dry matter.

The results of the determinations of gluten are given in Table III. It was impossible to wash out the glutens of the very heavily infected samples No. 441/0 and No. 441/6; during the washing out of these samples no gluten whatever was recoverable, and with several other samples it was only possible to recover gluten with difficulty.

### Kneading Tests (Farinograph)

In view of the foregoing it was only to be expected that there would be large differences among the various samples, and curves typical of such differences are given in Figure 1.

In the case of curves (farinograms) No. 441/0 and No. 441/6 (those samples yielding no gluten in the gluten washing test), it will be seen that after a kneading time of three to four minutes, in which the doughs attain a maximum consistency of 500 g., a sharp decrease in consistency is apparent. After a kneading time of ten minutes the drop in the consistency is 210 g. and 280 g. respectively. The condition of the dough at this point is sticky.

In contrast to the poor results on these inferior samples are the curves of samples No. 421/3 and No. 441/4, which are normal and indicate quite good and sound characteristics. Other curves range between the above two extremes.

<sup>1</sup>E. Berliner: "Leimkleberweizen" ist "Wanzenweizen," Mühlenlab. No. 4 (Mühle, Heft 32), 1931.



Fig. 1. Farinograph curves.

The kneading test has the advantage that it permits flours of even very inferior quality to be evaluated, and as these are made into dough in a mechanical dough mixer the examination can be made without the necessity of touching the dough with instruments or with the hand.

### Extensibility Tests

These tests, when using one or other of the well known testing devices, for example the Chopin Alveograph, the Brabender Extensograph, etc., are in contrast with the kneading tests, in as much as the doughs have to be taken out of the dough mixer and manipulated by hand for weighing and shaping the dough before the extensibility test can be made.

Thus it is very difficult, when making the test for extensibility, to handle and examine inferior flour doughs because of their stickiness. Consequently Nos. 441/0, 441/5, 441/6 (in which it was impossible or difficult to retain any gluten in the washing test) could not be so tested. Obviously this is an undesirable feature and is in itself indicative of very poor quality.

Other samples could be measured only after a dough rest of 45 minutes at a temperature of 27° C., but this was no longer possible after a period of 135 minutes. Samples falling into this category were Nos. 127R, 139, 402/3, 402/4, 402/5, 441/53, 431/4. Some of the curves are shown in Figure 2.

The following remarks apply to all the samples to which it was possible to apply the extensibility test either partly or completely.

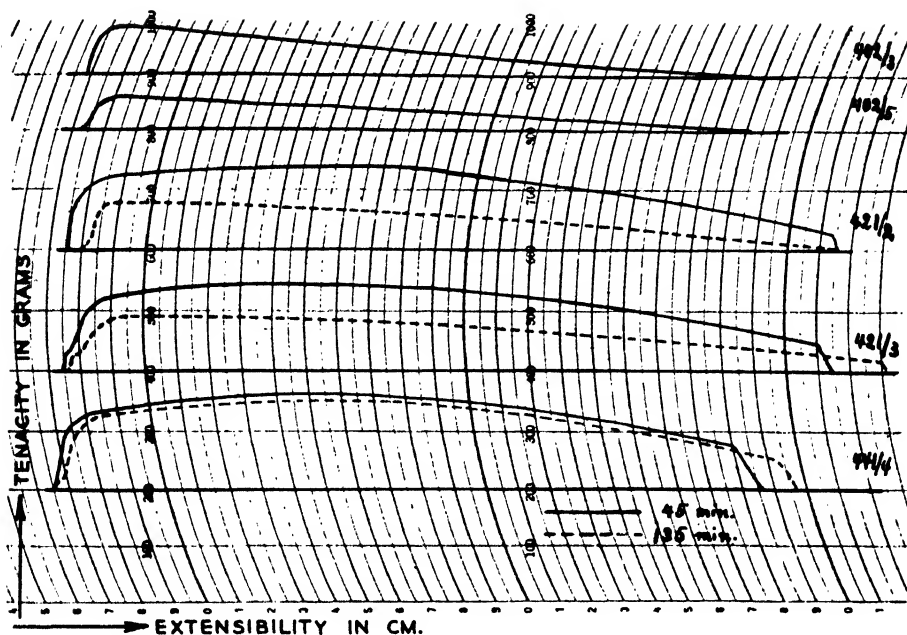


Fig. 2. Tenacity and extensibility of doughs (53% water on 15% basis).

The extensibility of the dough is poor, for the dough is very short. The elasticity leaves much to be desired, and this is shown by the abnormal diagram curves (see Figure 2, Nos. 402/3 and 402/5). The tenacity (resistance to extension) is often slight initially, and there is a big difference in tenacity between a dough rest of 45 minutes and a dough rest of 135 minutes at 27° C. (see curves for Nos. 421/2 and 421/3). Again it will be observed that sample No. 441/4 is foremost in quality.

### Baking Tests

The baking tests were carried out using water with 2% of salt and 2½% of yeast, the temperature of the dough being 27° C. and the water absorption calculated on a moisture content of 15% in the untreated flour. All flour samples were baked: (a) untreated, (b) treated with potassium bromate. Because of the treatment with potassium bromate the water absorption was increased by 1½% and in most cases an improvement in volume resulted. The poorer samples however were an exception and responded only slightly or not at all to treatment.

The results of the baking tests confirm the results of mechanical dough examination by kneading and extensibility tests. The wheats No. 402/3 and No. 402/5 were classified as very poor, while Nos. 441/0, 441/5, 441/6, 441/53 were classified as unsuitable for baking purposes.

TABLE IV  
BAKING TESTS

Wheat No.	Bread volume, 100 g. flour (untreated)	Bread volume, 100 g. flour (treated)	Water absorption	Remarks
	cc.	cc.	%	
127 R	507	657	56 $\frac{1}{2}$	
139	473	621	56 $\frac{1}{2}$	
402/3	378	588	56 $\frac{1}{2}$	(Dough weakened markedly, resultant loaf flat)
402/4	468	650	56 $\frac{1}{2}$	
402/5	384	574	54 $\frac{1}{2}$	(Dough weakened markedly)
421/2	532	667	56 $\frac{3}{4}$	
421/3	505	748	58 $\frac{1}{2}$	
421/32	625	701	56 $\frac{1}{2}$	
421/61	575	704	58 $\frac{3}{4}$	
431/3	384	627	54 $\frac{1}{2}$	
431/4	413	668	54 $\frac{1}{2}$	
431/61	420	706	55 $\frac{3}{4}$	
441/0	290	297	56	(Dough exceptionally bad, very slimy; loaf collapsed)
441/2	450	633	56 $\frac{1}{2}$	
441/4	606	672	56 $\frac{3}{4}$	
441/5	364	451	56 $\frac{1}{2}$	(Dough very bad, weakened markedly; loaf flat)
441/6	326	329	55 $\frac{1}{2}$	(Dough and loaf as 441/0, collapsed)
441/53	332	483	54	(Dough very bad, weakened markedly; loaf rather flat)

### Behavior of Russian Wheats when Used in a Blend

Normally when sound wheats are blended, the influence of any one kind on the quality of the grist as a whole is more or less in direct proportion to its own inherent qualities, and to the quantity used.

With Russian wheats affected by the wheat bug this was not the case, owing to the fact that the incorporation of a percentage of this infected wheat in the blend introduced an abnormally high percentage of proteolytic enzymes, whose destructive effects were very prominent in the flour and dough. Actually the presence of such Russian wheat in the grist affects the blend in two ways: (a) by the poor quality of its gluten, and (b) by its excess of proteolytic enzymes. The unfavorable effect was not confined in the grist to the Russian wheat itself but was transmitted to the other wheats in the blend, by virtue of its high protease content.

An investigation was made of the effect of introducing a percentage of No. 441/0 (gluten nonretainable in washing test) into the wheat mixture. This was carried out by adding flour milled from No. 441/0 to a straight-run control flour which had been milled from a wheat mixture normally used in Holland and consisting of Manitobas

(Canada), hard winter (U. S. A.), Plate (Argentina), and a percentage of home-grown (Netherlands) wheat, the ash content of this flour being 0.58%, calculated on dry matter. The resulting blended flour was examined mechanically by kneading and extensibility tests, using the Extensograph (Brabender) and the Comparator (Buhler).

The extensibility tests especially showed that an admixture of as little as 2% of No. 441/0 exerted a distinctly unfavourable influence on the quality of the flour.

TABLE V  
EXTENSIBILITY TESTS WITH EXTENSOGRAPH AFTER DOUGH RESTS OF  
45 AND 135 MINUTES AT 27°C.

(Dough was made using 53% water and 1.65% salt)					
Russian wheat flour 441/0, admixed	Dough rest of 45 minutes		Dough rest of 135 minutes		Difference in tenacity at 45 and 135 min.
	Tenacity	Extensi- bility	Tenacity	Extensi- bility	
	<i>g.</i>	<i>cm.</i>	<i>g.</i>	<i>cm.</i>	
0%	250	23½	250	21½	0
2%	250	22	235	21	15
4%	210	23½	180	23½	30
6%	205	23½	150	22½	55

The addition of flour milled from the infected Russian wheat obviously caused the tenacity of the flour to decrease; without any addition of such flour the tenacity of the control flour after dough rests of 45 minutes and 135 minutes was the same. With a small percentage of the affected flour added thereto (2%) the tenacity of the dough not only decreased after 45 minutes but to an even greater extent at 135 minutes. The drop in tenacity between 45-minute and 135-minute periods was not in proportion to the percentage of affected flour used, but such drop was in increasing proportion, for example:

A 2% admixture resulted in a drop in tenacity of 15 g. on 250 g. = 6%.  
A 4% admixture resulted in a drop in tenacity of 30 g. on 210 g. = 14%.  
A 6% admixture resulted in a drop in tenacity of 55 g. on 205 g. = 26%.

The results given in Table V were confirmed by extensibility tests made on the Comparator (Buhler). Repeat tests on the extensograph, this time with yeast doughs using 2½% of yeast, yielded similar and confirmatory results. Baking tests gave indications substantiating the mechanical tests given in detail above.

To complete the investigation a further series of tests was made by adding proportions of the affected Russian wheat flour to soft flours milled from home-grown wheat, and the tests in this series gave results similar to those of the tests made with the normal baker's grade of flour.

### Summary

The Russian wheat of the 1938 crop imported into the Netherlands was carefully examined. All parcels of Russian wheat of this crop were more or less damaged by the wheat bug. The protein determination of such wheats does not allow satisfactory conclusions as to the true baking value of these wheats.

In view of the foregoing it was necessary to grind every parcel of the wheat experimentally before incorporating such wheat into the wheat blend. Straight-run flour was therefore obtained on a test-milling plant, this flour having an ash content of 0.55%–0.58% and each of the flours so milled was subjected to mechanical examination of the flour followed by routine test bakings. Of the samples examined 25% were so badly affected that they were unsuitable for baking purposes. The remainder varied between moderate and fairly good in quality.

Extensibility tests of the doughs showed generally the lack of elasticity, and led to abnormal "curves" in mechanical tests. The extensibility of the dough was of moderate character, the doughs being rather on the short side.

The influence of badly affected Russian wheat flour on a good straight-run control flour was examined. It was found that an admixture of only 2% caused a measurable depreciation in the quality of the dough. The extensibility test was found to be extraordinarily sensitive and therefore very suitable for examinations of flours of this kind.

Of particular importance was the fact that the affected Russian wheat flour which was admixed in relatively small proportion was found to increase the proteolytic activity in the mixture out of proportion to what was anticipated, and owing to this the sound flour was badly affected, not in direct proportion to the amount added but in progressively increasing proportion. This was reflected in terms of decreasing tenacity of the dough when the resting time of the dough was increased.

# THE IMPORTANCE OF GAS-PRODUCTION AND GAS-RETENTION MEASUREMENTS DURING THE FERMENTATION OF DOUGH

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(Read at the Annual Meeting, May 1940)

The Report of the 1938-39 Committee on Methods of Analysis of the American Association of Cereal Chemists (Sandstedt, 1940) comprises among others the following recommendations: (a) that the study of methods for evaluating yeasts be continued, and (b) that the investigation of gas retention be continued.

Although numerous analyses and separate determinations have been proposed for the evaluation of flour strength and dough properties, an increasing number of cereal chemists believe with good reason that gas production and gas retention during the fermentation of dough are the most important factors of bread production. The writer has been astonished to find that the correctness of this opinion does not seem to be sufficiently recognized, and a short historical review of the literature, therefore, may support this thesis.

Nearly half a century ago, H. Elion (1893) drew attention to the fact that in the fermentation of dough different factors have such an influence on the dough volume that the quantity of gas produced becomes a factor of minor importance, and he pointed out that various other flour properties influence the rising of the dough during fermentation and in the oven.

As far as I am aware, Maurizio (1902) was the first to publish extensive experiments which made a clear differentiation between gas-production and gas-retention capacities. Wood (1907), using the device published previously by H. Elion (1903) (see E. Elion, 1933), confirmed Maurizio's statements.

Various definitions of flour strength (Jago, 1895; Humphries and Biffen, 1907; Wood, 1907; Humphries, 1907; Humphries and Simpson, 1909) also lead to a clear differentiation between gas production and gas retention and to suggestions that these factors be considered as components of strength.

Numerous papers dealing with the importance of gas production and gas retention have appeared during the last three decennia. I refer only to Bailey (1916), Bailey and Weigley (1922), Bailey and Johnson (1924), Blish and Sandstedt (1927), Working (1929), St. John and Bailey (1929), Fisher and Halton (1929, 1929a), Jørgensen (1931), Markley and Bailey (1932), Blish and Hughes (1932), Geddes and



Larmour (1933), Larmour and Brockington (1934), Kosmin (1936, 1937), Clark (1938), Kent-Jones (1937, 1939), and Bailey (1939).

From these papers it is evident that gas production and gas retention are the two factors that predominate in determining the character of the bread. Brabender (1933, 1934) stressed the importance of *properly balanced* gas-production and gas-retention capacities. He pointed out that dough development and gas production both reach a maximum at certain moments during the fermentation of the dough, and that in order to obtain the best baking results, the moments when these maxima are reached should coincide. As a matter of fact, good gas-production and gas-retention capacities are indispensable for good bread, but even with both the resulting loaf may be unsatisfactory, if the dough development starts too late or too early with regard to the available gas production. A good gas production during the fermentation of the dough must, therefore, be accompanied by a good gas-retention capacity during the period when the gas production is available.

From the foregoing one may conclude that the measurement of both gas production and gas retention *during the whole course of fermentation* must be considered as extremely important, as well for the flour miller as for the baker.

Whereas several devices for the measurement of gas production have long been available, this is not the case for gas retention, and this may be one of the reasons that a number of the studies mentioned above have been made with baking tests, in which the personal factor plays an important role. Markley and Bailey (1932), discussing previous nonautomatic methods for the determination of the relation between the production of gas in and loss of gas from a fermenting dough, state that these methods are laborious, requiring practically the constant attention of the technician, and they propose an automatic gas-production registering mechanism, the saving in time of the operator justifying the added cost of the entire device.

In recent years several recording apparatuses for flour research have been constructed in order to eliminate the personal factor involved with baking tests (see E. Elion, 1939). As far as gas production is concerned Brabender's fermentograph has claimed attention. Schmalz and Sullivan (1938), however, pointed out some errors involved in the measurement of gassing power by this device, and stated that several errors can be overcome and compensated for, except a loss of gas by diffusion through the rubber balloon, which, according to these authors, is too large an error to be disregarded.

Another recording device for gas production is the S. I. A. Fermentation Recorder. It is said that this instrument, although not designed for it, can be modified to measure gas retention too. The au-

thor does not have access to any published data obtained with this apparatus.

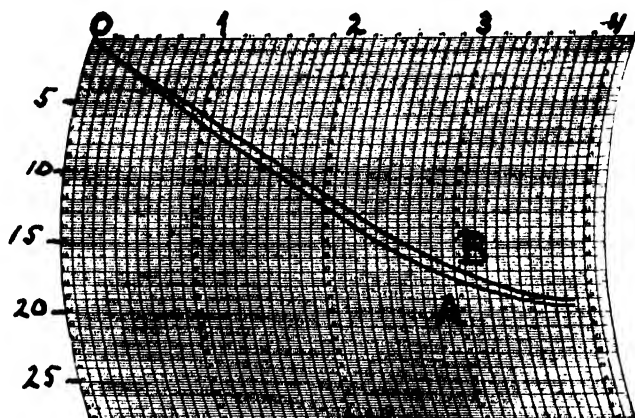
The complexity of the problem of gas retention may be one of the reasons for the lack of recording devices for its measurement. As a matter of fact, in efforts to approach the problem from other sides, several instruments have been developed recording only separate dough properties during short periods of the fermentation. Such separate dough properties, however, are but more or less related to baking quality and the interpretation of the experimental results and their correlation with practical baking may offer difficulties. As a general objection to all devices which record only one single dough property during a short period of the fermentation, such as various machines for physical tests of flour dough, I quote Munz and Brabender (1940), who recently stated: "Dough properties change steadily with time and as a function of mixing, fermentation, and other treatments. It appears important, therefore, to study the rate and direction of these changes rather than to measure dough properties at one stage only." Furthermore, such machines have been designed only for the study of one dough property, and consequently they may fail to demonstrate other very important properties, for the determination of which one must use other recording devices if a more complete picture of the dough properties is to be obtained.

G. Mueller (1935) discusses a number of methods which have been recommended for the determination of separate properties, such as gluten quality, gluten quantity, and extensibility of dough, and he also points out the errors connected with determinations of single properties of this kind.

As stated above, the two essential factors that predominate in determining flour quality and the character of bread are gas production and gas retention. It is obvious that very useful information can be obtained by a device that records exactly these two properties and therefore can replace several other devices recording different individual properties respectively, which together contribute to gas-production and gas-retention capacity. An apparatus which complies with these requirements, *i.e.* which records automatically both gas production and gas retention during the whole course of fermentation, is actually available under the name Chefaro Balance. This device, which has found increasing application in European countries, has been developed by the Chemische Fabriek Rotterdam, of Rotterdam, Holland, and has been described by E. Elion (1939, 1940). It consists of two special precision balances, by which the quantities of gas actually produced by or retained in the fermenting dough are accurately weighed and automatically recorded during the whole course of the fermentation.

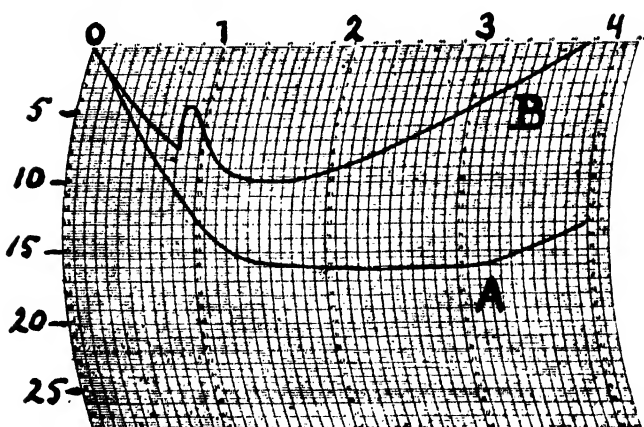
Sources of errors connected with other methods have been eliminated and many advantages are present.

Carbon dioxide is collected in a special gas-jar, which is absolutely gas-tight, so that no gas can escape. The dough is surrounded by air



Horizontal: Duration of fermentation in hours.

Vertical: Quantity of gas developed in cubic centimetres (doughs of 1 gram of flour). -



Horizontal: Duration of fermentation in hours.

Vertical: Quantity of gas retained in cubic centimetres (doughs of 3 grams of flour). -

Fig. 1. Gas-production and gas-retention curves as indicators of flour quality.

during the fermentation, as in the bakery, and no materials such as rubber are used. The apparatus records automatically the real gas production and gas retention, *i.e.* the combined effect of numerous known and unknown separate properties, instead of some arbitrary single properties only. The fermenting dough is permanently visible. The time re-

quired to start an experiment is short. The device works automatically and does not require further attention or readings. For large-scale operations several balances can easily be operated by one person. The automatic recording has proved to be a saver of time for the operator. The Chefaro Balance does not require a skilled person for its operation, because faulty determinations can hardly occur. After some little practice excellent duplicate results can be obtained. Figure 1 demonstrates the importance of obtaining both gas-production and gas-retention curves.

There is hardly any difference between the gas production of flours *A* and *B*, both of which produce about 19 cubic centimeters of fer-

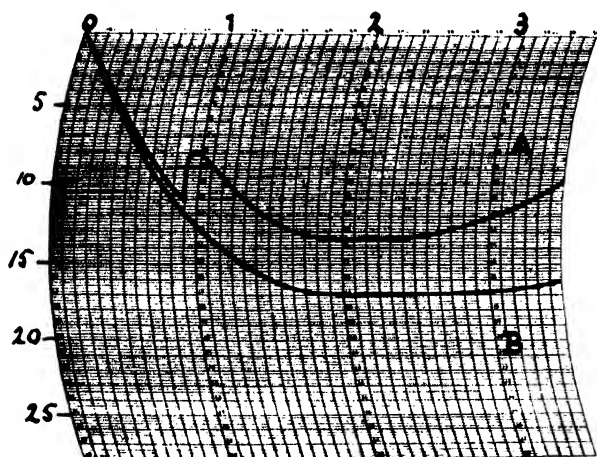


Fig. 2. Gas-retention curves showing effect of flour improvers.

mentation gas (from one gram of flour). In each case fermentation lasts about three hours and a half. Nevertheless it appears that the quality of the two flours differs greatly. Flour *A* gives a loaf volume of 1160 cc., whereas flour *B* only attains a volume of 790 cc.

When examining the gas *retention* capacities, the difference in baking value is soon explained, because a dough made from flour *A* is able to retain 16.2 cc. of fermentation gas as compared with only 10.2 for flour *B*. Moreover the stability (or fermentation tolerance) of flour *A* is much better, the maximum gas-retaining capacities of the doughs *A* and *B* being maintained for 80 and 25 minutes respectively. The gas-production and gas-retention capacities of flour *B* are not properly balanced, as is proved by the temporary escape of gas after 50 minutes.

A further considerable advantage of the Chefaro Balance, which is missing in many of the devices recording separate physical dough properties, consists in the fact that it records the influence of flour improvers and baking agents on gas production and on gas retention (Fig. 2).

An untreated flour (curve *A*) has a maximum gas-retaining capacity of 13.3 cc. A treatment by 12 parts of flour improver to every 100,000 parts of flour strengthens the gluten to such an extent that 17.1 cc. of gas are retained (curve *B*), representing an increase of 28.5%.

The curves show furthermore that the dough stability is much improved by the treatment. The strengthened dough supports the gas pressure longer and therefore may be expected to show better resistance to bakeshop handling.

Since the Chefaro Balance records the influence of flour improvers, this instrument is especially competent to determine the quantity of any improver that will give the best results with a given flour (Fig. 3).

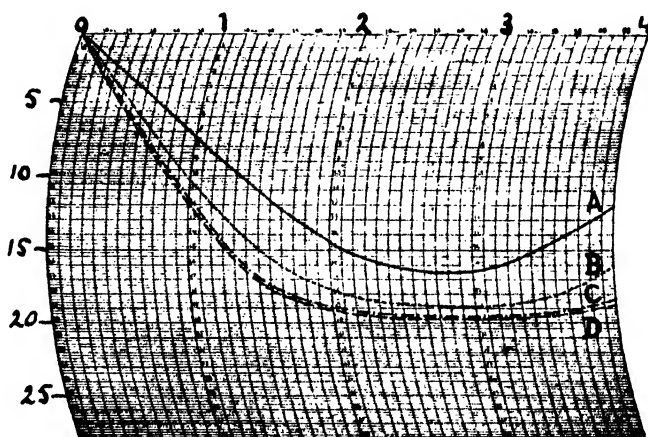


Fig. 3. Variations in gas retention with varying concentrations of improver.

Curve *A* = untreated flour.

Curve *B* = treated with 10 parts of improver to 100,000 parts of flour.

Curve *C* = treated with 12 parts of improver to 100,000 parts of flour.

Curve *D* = treated with 15 parts of improver to 100,000 parts of flour.

The duration of fermentation being recorded on the horizontal axis, it may be observed that the dough when so treated attains its full development more rapidly and that the maximum expansion is maintained for a much longer period.

The gas retention is recorded in a vertical direction and this capacity is increased from 16.5 cc. to 19.7 cc., *i.e.* 3.2 cc. or 19.4%. Further, the graphs show that the treatment with 12 parts of improver is most suitable, the small differences between the graphs *C* and *D* not justifying an increase of 25% in the cost of treatment.

Numerous other applications can be made of the Chefaro Balance, for instance, in studying the influence of the maturing effect of flour storage, different dough mixing methods and different mixing speeds, different yeasts, etc.

When considering the number of published reports of studies on

gas production and gas retention, it may be concluded that the knowledge of these two important factors in bread making could have advanced more rapidly if the Chefaro Balance had been available earlier; and in line with the recommendations of the 1938-39 Committee on Methods of Analysis of the A. A. C. C., mentioned above, the author believes that the application of this new device may prove to be very useful.

### Summary

The author cites numerous publications which demonstrate that gas production and gas retention are now generally recognized as the two essential factors that predominate in determining flour quality and the character of bread. He points out that methods which determine only separate properties belonging to the complex problem of gas retention may be of questionable value in the study of flour properties and he stresses the logic and desirability of recording automatically the actual gas production and gas retention during the whole period of dough fermentation, as has been made practically possible by the development of the Chefaro Balance. This instrument consists of two special precision balances, by which the quantities of gas actually produced by or retained in the fermenting dough are accurately weighed and automatically recorded during the whole course of fermentation.

In consequence of some of the advantages and applications of this new device, its further use in the study of gas production and gas retention is recommended.

### Literature Cited

- Bailey, C. H.  
1916 A method for the determination of the strength and baking qualities of wheat flour. *J. Ind. Eng. Chem.* **8**: 53-57.  
1939 Measuring fermentation rate and gas losses in dough. *Cereal Chem.* **16**: 665-671.  
— and Johnson, A. H.  
1924 Carbon dioxide diffusion ratio of wheat flour doughs as a measure of fermentation period. *Cereal Chem.* **1**: 293-304.  
— and Weigley, Mildred  
1922 Loss of carbon dioxide from dough as an index of flour strength. *J. Ind. Eng. Chem.* **14**: 147-150.  
Blish, M. J., and Hughes, R. C.  
1932 Some effects of varying sugar concentrations in bread dough on fermentation by-products and fermentation tolerance. *Cereal Chem.* **9**: 331-356.  
— and Sandstedt, R. M.  
1927 Factors affecting interpretation of experimental baking tests. *Cereal Chem.* **4**: 291-299.  
Brabender, C. W.  
1933 Vier Jahre Farinograph und Fermentograph. *Mehlprobleme* **2**: 47-49.  
1934 Six years farinography. *Cereal Chem.* **11**: 586-597.  
Clark, R. J.  
1938 The meaning of fermentation tolerance. *Cereal Chem.* **15**: 342-344.

- Elion, E.  
1933 A simple volumetric method for measuring gas production during dough fermentation. *Cereal Chem.* 10: 245-249.  
1939 Some remarks on the varying influence of compressed yeasts of different industrial origin on the gas retention of dough, as recorded by a new instrument, the Chefaro balance. *Cereal Chem.* 16: 598-610.  
1940 The Chefaro balance for the automatic measurement of gas production and gas retention during the fermentation of dough. *The Baker's Digest* 14: 143-147, 151.
- Elion, H.  
1893 Studien über Hefe. *Centr. Bakt. Parasitenk.* 14: 53-62, 97-108.  
1903 Broodgisting. *Handelingen 9e Nederl. Natuur- en Geneesk. Congres.*
- Fisher, E. A., and Halton, P.  
1929 The relation of hydrogen-ion concentration and buffer value to the baking quality of flour. Part II. *Cereal Chem.* 6: 97-115.  
1929a A correction. *Cereal Chem.* 6: 504-505.
- Geddes, W. F., and Larmour, R. K.  
1933 Some aspects of the bromate baking test. *Cereal Chem.* 10: 30-72.
- Humphries, A. E.  
1907 Causes of the quality strength in wheaten flour. *Chem. News* 96: 85.  
— and Biffen, R. H.  
1907 The improvement of English wheat. *J. Agr. Sci.* 2: 1-16.  
— and Simpson, A. G.  
1909 Gas-making capacity as a factor in the estimation of strength in wheaten flours. *Seventh Int. Congr. of Appl. Chem., London, Section VIA.* 27-33.
- Jago, W.  
1895 A text-book of the science and art of bread-making. London.
- Jørgensen, H.  
1931 On the separation of "gassing power" (diastatic activity) from "strength" in baking tests. *Cereal Chem.* 8: 361-374.
- Kent-Jones, D. W.  
1937 Progress of cereal chemistry. *Livre Jubilaire, Institut Supérieur des Fermentations, Gand.* 41-44.  
1939 Modern cereal chemistry (3rd ed.). The Northern Publishing Company, Liverpool.
- Kosmin, Natalie P.  
1936 Das Problem der Backfähigkeit. *Verlag von Moritz Schäfer, Leipzig.*  
1937 Das Problem der Backfähigkeit des Weizenmehls. *Livre Jubilaire, Institut Supérieur des Fermentations, Gand.* 65-74.
- Larmour, R. K., and Brockington, S. F.  
1934 Studies on experimental baking tests. I. Effects of variation in baking formulas on gas production and loaf volume. *Cereal Chem.* 11: 451-486.
- Markley, M. C., and Bailey, C. H.  
1932 An automatic method for measuring gas production and expansion in doughs. *Cereal Chem.* 9: 591-594.
- Maurizio, A.  
1902 Die Backfähigkeit des Weizens und ihre Bestimmung. *Landw. Jahrb.* 31: 179-234.
- Mueller, G.  
1935 Welche der heutigen Methoden zur Bestimmung der Weizenqualität sind für eine gerechte Weizenstandardisierung brauchbar? *Mehlprobleme* 4: 29-39, 42-50.
- Munz, E., and Brabender, C. W.  
1940 Prediction of baking value from measurements of plasticity and extensibility of dough. I. Influence of mixing and molding treatments upon physical dough properties of typical American wheat varieties. *Cereal Chem.* 17: 78-100.
- Sandstedt, R. M.  
1940 Report of the 1938-39 committee on methods of analysis. *Cereal Chem.* 17: 129-130.
- Schmalz, F. D., and Sullivan, B.  
1938 Errors involved in the measurement of gas production by the fermentograph. *Cereal Chem.* 15: 409-413.

- St. John, J. L., and Bailey, C. H.  
1929 Effect of dry skim milk on the fermentation and hydrogen-ion concentration of doughs. *Cereal Chem.* 6: 51-59.
- Wood, T. B.  
1907 The chemistry of the strength of wheat flour. *J. Agr. Sci.* 2: 136-160, 267-277.
- Working, E. B.  
1929 Fermentation tolerance. *Cereal Chem.* 6: 506-512.

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## THE USE OF HAND-OPERATED SHEETING ROLLS IN TEST BAKING<sup>1</sup>

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(Read at the Annual Meeting, May 1939)

The entire or partial mechanization of the experimental baking test has occupied the interest of cereal technologists for a considerable period of time, and a rather extensive literature pertaining to this subject already exists. A recent paper by Heald (1939) reviews most of the important contributions relative to this subject. The use of sheeting rolls in connection with dough molding has been specifically discussed by Merritt, Blish, and Sandstedt (1932), Geddes and Sibbitt (1933), and Heald (1939). However, the need for additional information and discussion is necessitated by the fact that there is now available, at reasonable cost, a regularly manufactured sheeting roll which is coming into more general use than any previous similar piece of equipment. It is of interest therefore to determine the suitability of this particular type of sheeting roll for use in conducting the baking test.

### Experimental

The information presented in this paper deals with the use of the hand-operated sheeting roll manufactured by The National Manufacturing Company, Lincoln, Nebraska. In the study here reported, the rolls were used to sheet the dough previous to molding and were not used during the punching procedure. The method of molding involved removing the dough from the fermentation bowl with the hands slightly greased and placing it on a canvas-covered board. The dough was gently flattened and slightly elongated before being put through the sheeting rolls. The scanty film of grease which the dough collects from the hands and canvas prevents its sticking to the roll surface without the need of greasing the roll or supplying dusting flour.

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<sup>1</sup> Subcommittee report, 1938-39 Committee on Standardization of Laboratory Baking.



In order to obtain the desired dough sheet without causing a tearing of the gluten strands, the strong, hard-wheat doughs were put through the sheeter twice: once with a roll spacing of 5/16 inch and again with a setting of 7/32 inch. The pliable soft-wheat doughs were run through the sheeter only once, with the former setting. The sheeted doughs were placed in a wooden trough, of the same width as the bottom of the baking pan, and rolled up fairly tight. The trough prevents the doughs from becoming elongated during the molding process, eliminates the necessity for sealing the dough ends, and allows the operator to introduce easily the doughs into the baking pans. The high pan type was used in this study.

Data for the statistical analysis of the variability of loaf volumes and bread scores between bread molded by hand, in the manner prescribed in the A. A. C. C. Book of Methods (1935), as contrasted with the bread produced by the procedure outlined above, were obtained by making a series of bakes using both a hard and a soft wheat flour. Sixty-five bakes using both methods, with all other conditions identical, were secured using the hard wheat flour; and fifty comparisons were made using the soft wheat flour.

### Results

An inspection of Table I indicates that the standard deviations of the loaf volumes for the hard wheat flour were practically identical. A

TABLE I  
VARIABILITY OF LOAF VOLUME

Series	Molding method	Type of flour	No. of variates	Mean loaf volume	Standard deviation	Coefficient of variation
A	Hand	Hard	65	665	22.2	3.3
B	S-Rolls	Hard	65	656	22.6	3.4
C	Hand	Soft	50	444	17.3	3.9
D	S-Rolls	Soft	50	442	10.8	2.4

value of 22.2 was obtained by the hand method and a value of 22.6 when the sheeting rolls were used. The use of sheeting rolls on the soft wheat flour reduced the standard deviation from 17.3 to 10.8. The loaf volumes were slightly larger for both flours when molded by hand.

All the bread was scored by assigning numerical values to the important external and internal characteristics of the loaves, including the volume.

In Table II is recorded the variability of the bread scores for both flour types. The use of sheeting rolls does not offer any outstanding

TABLE II  
VARIABILITY OF BREAD SCORE

Series	Molding method	Type of flour	No. of variates	Mean loaf score	Standard deviation	Coefficient of variation
A	Hand	Hard	65	93.8	1.6	1.7
B	S-Rolls	Hard	65	94.7	1.5	1.6
C	Hand	Soft	50	82.7	1.2	1.5
D	S-Rolls	Soft	50	83.3	1.1	1.3

advantages from the standpoint of variability in the replication of results but they do give a better uniformity of grain and texture than did loaves molded by hand. This fact accounts for the higher average bread score when the sheeting rolls were employed.

### Summary and Conclusions

A series of bakes were conducted, using a hard and a soft wheat flour, in which alternate doughs were handled with and without the use of sheeting rolls during the molding process. When both operations were conducted in the same laboratory by experienced operators, sheeting rolls did not offer any outstanding advantages although they did tend to reduce the variability in the replication of results.

The use of sheeting rolls slightly reduced the loaf volumes and increased the bread scores. This latter result is due to better uniformity of grain and texture.

Sheeting rolls certainly merit careful consideration as an additional step toward the complete standardization of the A. A. C. C. baking test for bread.

### Literature Cited

- Geddes, W. F., and Sibbitt, L. D.  
1933 Variability in experimental baking. IV. Cereal Chem. 10: 560-584.  
Heald, W. L.  
1939 Hand punching and hand molding vs. machine punching and machine molding. Cereal Chem. 16: 24-29.  
Merritt, P. P., Blish, M. J., and Sandstedt, R. M.  
1932 Report of activities of the A. A. C. C. baking research fellowship. Cereal Chem. 9: 175-238.

# THE AUTOLYTIC DIGESTION OF FLOUR IN RELATION TO VARIETY AND ENVIRONMENT

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Factors which influence the supply of gas in a dough are of great interest and importance to the wheat miller and baker. It is proposed, in this paper, to discuss and produce evidence concerning the effect of variety of wheat and environment during growth on the potential gas production of Australian wheat flour. Since it is not always expedient to bake a sample of flour to evaluate its gassing possibilities, other tests such as can be carried out with small portions of dough or water suspensions of flour are substituted. The latter and most common of the methods employed involves the determination of the sugar formed during an autolytic digestion of ten grams of flour at a given temperature for one hour (American Association of Cereal Chemists, 1935; Kent-Jones, 1927). The result is referred to as either the "diastatic activity" or "maltose figure" and is expressed as milligrams of maltose per ten grams of flour or as percentage of maltose formed. The emphasis in this paper is transferred from the amount of gas which could be produced, to the material from which it originates, namely the sugar, maltose.

The maltose produced during the autolytic digestion of a flour has been shown to be influenced by the variety of wheat from which the flour was made (West, 1932; Markley and Bailey, 1934; Swanson, 1935; Mangels, 1936; Hickinbotham, 1936a, 1936b). The environment of the wheat during growth and harvesting (Bailey, 1925; Mangels, 1926, 1934; Markley and Bailey, 1934; Swanson, 1936; Hickinbotham, 1936b; Farquhar, 1938; Breakwell, 1938; Breakwell and Hutton, 1939) and the milling treatment have also been found to exert an influence on the maltose figure (Johnson, 1930; Pascoe, Gortner, and Sherwood, 1930; Karacsonyi and Bailey, 1930; Markley and Bailey, 1934; Swanson, 1935; Gründer, 1935; Geddes and Aitken, 1935; Hickinbotham, 1936b; Leatherock, McGhee, and Giertz, 1937; Pulkki, 1938; Ståmberg and Bailey, 1939a).

Since there are two factors, substrate and enzyme, concerned in the maltose production as determined by an autolytic digestion, it is of interest to know whether the influences of variety, environment, and milling are mainly on the substrate, on the enzyme, or on both.

Variation in the enzyme factor between different flours may be a result of difference in amounts and/or kinds of amylase. At present

two or probably three amylolytic enzymes capable of attacking uncooked starch are considered to be present in wheat flour (Sandstedt, Blish, Mecham, and Bode, 1937; Blish, Sandstedt, and Mecham, 1937; Hanes, 1937). These are known as alpha- and beta-amylase, and the enzyme capable of attacking raw starch is referred to as the "raw starch factor." Those flours which are made from ungerminated sound wheat contain beta-amylase with or without the raw starch factor. Flour made from sprouted wheat contains those enzymes present in flour made from sound wheat and in addition alpha-amylase (Sandstedt, 1938). There may be still some doubt as to the separate entity of the raw starch factor, inasmuch as it has been studied only in a mixture with alpha-amylase.

Beta-amylase, so far as is known, is present in appreciable but varying amounts, alpha-amylase is either absent or present in varying amounts, and the raw starch factor ranges in amount from little or none to appreciable quantities (Sandstedt, Blish, Mecham, and Bode, 1937; Blish, Sandstedt, and Kneen, 1938). Estimations of the amounts of the various enzymes have been carried out by modifications of the Lintner procedure (American Association of Cereal Chemists, 1935; Sandstedt, Blish, Mecham, and Bode, 1937; Blish, Sandstedt, and Kneen, 1938; Collatz and Racke, 1925; Malloch, 1929; Hills and Bailey, 1938) involving partial or complete extraction of the enzymes and evaluation of the amount by allowing either the extract or a purified preparation of it to act on a given substrate. Beta-amylase is able to convert cooked starch or starch granules which have been thoroughly disrupted in a rod mill (Stamberg and Bailey, 1939b) to maltose at a rate depending on the enzyme concentration. Beta-amylase will convert up to about 60 % of cooked starch to maltose (Hanes, 1937; Blish, Sandstedt, and Kneen, 1938). The residual dextrin material (40 %) termed erythrodextrin can be completely converted to a lower dextrin by alpha-amylase if the enzyme concentration is high enough. The raw starch factor is able presumably to convert the raw uninjured starch granules into a dextrin or maltose, the rate of conversion depending on the concentration of the enzyme (Sandstedt, Blish, Mecham, and Bode, 1937; Blish, Sandstedt, and Mecham, 1937; Blish, Sandstedt, and Kneen, 1938).

The various combinations of the amylolytic enzymes as they occur in flour offer a complex problem to the cereal chemist and one finds the interpretation of their part in the autolytic digestion of flour rendered more difficult because of the nature of the substrate on which they act.

It has been shown that the susceptibility of the starch to amylases varies in different flours (Alsberg, 1927; Mangels, 1926; Hermano and Rask, 1926; Malloch, 1929; Andrews and Bailey, 1934; Sandstedt,

Blish, Mecham, and Bode, 1937; Blish, Sandstedt, and Mecham, 1937; Eva, Geddes, and Frisell, 1937; Harris and White, 1938; Blish, Sandstedt, and Kneen, 1938), durum wheat starch being most susceptible and starch from soft-wheat flour being the least susceptible to attack. This difference may be considered to be possible of explanation in two ways. Either the raw starch granules are uniform in composition in a given sample and bear within their structure or composition some property, the alteration of which, by heredity or environmental conditions, results in a change in susceptibility of each granule of that particular starch, or the individual starch granules are not uniform but consist of a susceptible group and a resistant group. Varying amounts of each group would then give rise to differences in susceptibility for the samples.

The work done by Sandstedt, Blish, Mecham, and Bode (1937) has shown that the susceptibility of the raw uninjured starch in different samples of amylase-free flours, to an amylase containing extract of a given flour, is the same and that differences noted between samples of such inactivated flours are due to the varying proportions of the susceptible starch material which they contain.

The visible detection of starch granules which are susceptible to amylase action has been carried out by Brown and Heron (1879). More recent work by Pulkki (1938) has shown that it is possible to differentiate wheat-starch granules of two kinds by staining with iodine and congo red so that one class is coloured blue and the other partially or completely red. The latter class includes all the visibly injured granules and in addition a few other granules showing no discernible injury. Pulkki found that for a given sample of semolina and middlings material, the granules staining completely or partially red were practically all in the size class of  $20\mu$  diameter or over and that the percentage of these granules in that class is closely correlated with the fineness of the sample and with the percentage of maltose formed during autolytic digestion. He further showed that a strong malt extract acting on a sample of wheat starch resulted in a greater visible damage to each of the granules which was capable of being stained with congo red, but no increase in the total number of such granules.

### I—Susceptible Starch in Australian Flours

Flours milled from Australian-grown wheats vary widely in their maltose figures (Jewell, 1935; Hickinbotham, 1936a, 1936b; Farquhar, 1938; Breakwell, 1938; Breakwell and Hutton, 1939; Bottomley, 1938), a large proportion being lower than is considered desirable. The so-called "strong" or "hard" wheats such as Pusa 4, Baringa, and

Dundee usually give a high and satisfactory maltose figure, while the "weak" or "soft" wheat varieties such as Nabawa, Free Gallipoli, and Ford give a low maltose figure.

This lack of ability to produce maltose during an autolytic digestion and its indication of the probable inadequate gassing power of the flours has been compensated for by the miller and baker through the use of sprouted-wheat supplements, and by a certain amount of alteration in milling practice.

The method of staining used by Pulkki (1938), slightly modified as noted below, seemed to offer an approach to the study of the maltose figure in relation to the susceptible starch in different samples of flour, as contrasted with that relationship in a single sample of flour ground to varying degrees of fineness.

This section of the report concerns itself with the relation of the amount of susceptible starch to variety and locality where the wheat was grown, to the maltose figure from the autolytic digestion, to severity of grinding, to protein content, and finally with the effect of injury of starch granules on the density of the granules as revealed by sedimentation experiments.

### Experimental

Samples of flour made from the wheat varieties Baringa, Pusa 4, Nabawa, Free Gallipoli, and Ford, grown at three places in Victoria, and from Baringa, Pusa 4, Dundee, Nabawa, and Ford, grown at three places in New South Wales, were examined. Unfortunately not all varieties could be obtained from each place but sufficient were secured to indicate some very interesting relationships.

The Victorian flour samples were obtained from wheat supplied and milled by the Victorian Department of Agriculture. The wheat was first reduced in a Wiley mill. This stock was put through the reduction rolls of an experimental mill and sieved. The white stock was put through the reduction rolls a second time and sieved. The flour yield was 50% to 60%.

The New South Wales flour samples were obtained from the Department of Agriculture of New South Wales and were portions of experimentally milled flours prepared for use in that department.

Starch granules for staining were prepared from 10 g. of flour by washing out with water. The starch suspension was filtered through a fine silk sieve (13xxx). One-fourth of the suspension was diluted to 200 ml. One ml. of a 2% alcoholic iodine solution was added and, after 30 minutes, 5 ml. of a 1% aqueous solution of congo red was added. By the use of a projection microscope the images of the starch granules were projected on a ruled card where measurements were made with

a glass ruler at 500 magnification. Two classes of granules measuring  $20\mu$  or over in diameter were counted, those that stained partially or completely red and those staining blue. At least 3400 granules were measured for each sample.

The results are set out in Table I and indicate that there is a marked relation between the percentage of starch granules which stain with congo red and variety of wheat but no relation with the place where the wheat was grown.

TABLE I

THE PROPORTION OF STARCH GRANULES OF  $20\mu$  DIAMETER OR OVER WHICH STAIN RED WITH IODINE-CONGO RED SOLUTION

Place	Baringa	Pusa 4	Nabawa	Ford	Dundee	Free Gallipoli
	%	%	%	%	%	%
Dookie, Vic.	20.7	20.4	12.1	7.7	—	10.8
Werribee, Vic.	20.2	26.8	11.6	6.5	—	11.5
Walpeup, Vic.	23.2	20.6	10.3	7.2 <sup>1</sup>	—	10.7
Temora, N. S. W.	25.0	17.6	8.8	7.5	15.6	—
Gilgandra, N. S. W.	26.7	19.9	6.9	5.0	12.7	—
Condobolin, N. S. W.	26.1	17.1	10.3	9.3	17.3	—

<sup>1</sup> Value supplied by calculation.

Autolytic digestion by the Blish and Sandstedt method (American Association of Cereal Chemists, 1935) produced amounts of maltose in the different samples which bore a marked relation to variety and a slight tendency to be higher for the Victorian samples than for the New South Wales samples. (See Table II.)

TABLE II

THE DIASTATIC ACTIVITY—AS MALTOSE PER 10 G. OF FLOUR IN 1 HOUR AT 30°C.

Place	Baringa	Pusa 4	Nabawa	Ford	Dundee	Free Gallipoli
	mg.	mg.	mg.	mg.	mg.	mg.
Dookie, Vic.	245	233	165	164	—	208
Werribee, Vic.	233	279	156	118	—	143
Walpeup, Vic.	244	219	148	96 <sup>1</sup>	—	150
Temora, N. S. W.	258	239	118	96	203	—
Gilgandra, N. S. W.	336	235	103	66	—	—
Condobolin, N. S. W.	258	222 <sup>1</sup>	112	84	—	—

<sup>1</sup> Values supplied by calculation.

For purposes of the analysis of the results, the flour samples of the four varieties Baringa, Pusa, Nabawa, and Ford grown at each of the six places were considered. The missing values (3) in Tables I and II were supplied by calculation. The total correlation, +.934, represents the overall relationship between the two factors of percentage of starch

granules which stain with congo red and the diastatic activity for all the samples in Tables I and II. By eliminating all samples of Free Gallipoli and Dundee from consideration, the overall correlation becomes  $+0.954$  (see Table III).

TABLE III  
THE ANALYSIS OF VARIANCE AND CO-VARIANCE

	Degrees of freedom	Sums of squares and products			<i>r</i>
		<i>SX</i> <sup>2</sup>	<i>SY</i> <sup>2</sup>	<i>SXY</i>	
Total	21	1262.79	125,533	12,011.8	$+0.954$
Varieties	3	1136.60	107,801	11,060.3	$+0.999$
Places	5	7.61	3,151	30.3	$+0.196$
Varieties $\times$ places	13	118.58	14,581	921.2	$+0.701$

This is in reality a composite of each of the effects of their separate constituents. The two correlations  $+0.999$  and  $+0.196$  represent the values for the relationship between the two factors for the variety means and place means. The variety value is high and significant but it is only calculated on the basis of four means. The place value is low and not significant and therefore place of growth has little or no effect on the correlation between the percentage of granules staining red with congo red and the diastatic activity. The final correlation  $+0.701$  represents the relationship between these two factors after the variety and place effect has been removed. It is fairly high and significant, thereby indicating that apart from any variety effect there is a definite relationship between the two factors, diastatic activity and percentage of granules staining red in congo red solution.

The relationship between the percentages of granules stained with congo red and the maltose figures is set out graphically in Figure 1. The results given by Pulkki (1938) which are applicable for comparison purposes are also included in Figure 1. It is noteworthy that they show a similar relation between the maltose figures and percentages of granules stained with congo red. Those varieties such as Baringa, Pusa 4, and Dundee, requiring a high roller pressure to reduce the endosperm, have a high proportion of granules which stain with congo red and a high maltose figure. Other varieties such as Free Gallipoli, Nabawa, and Ford, which require less pressure, show few granules which stain with congo red and a low maltose figure. This constitutes evidence that it is the action of the milling processes on wheats with different inherent properties that is responsible for the variation in the maltose figure and the percentage of starch granules susceptible to staining with congo red.

As confirmation of Pulkki's work (1938), two samples of commercial



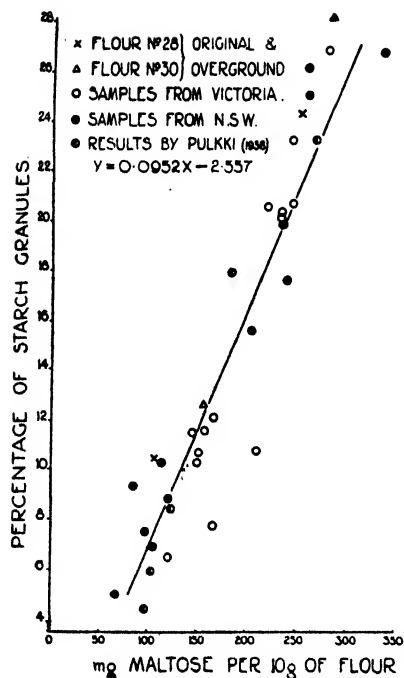


Fig. 1. The relation between the maltose formed after one hour at 30°C. and the percentage of starch granules of 20 $\mu$  diameter or over which stain with congo red.

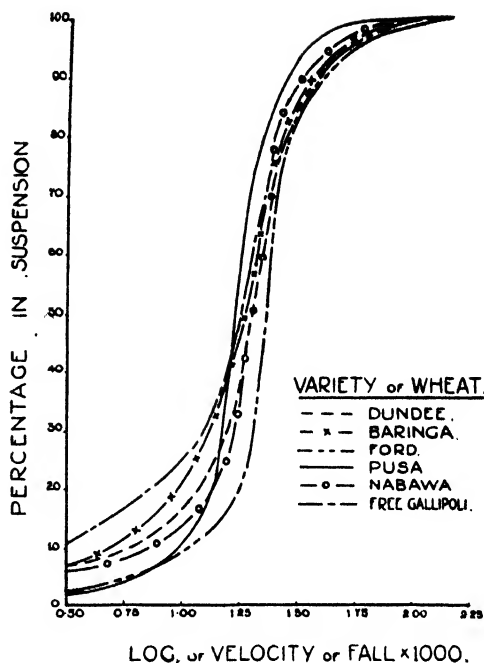


Fig. 2. Summation curves illustrating the sedimentation of starch granules from six varieties of wheat grown in Victoria, 1936.

flour were subjected to severe grinding by passing them several times through the closely set reduction rollers of an experimental mill. The data as set out in Table IV indicate the magnitude of increase both in the maltose figure and in the percentage of granules staining with congo red. The data are recorded in Figure 1.

TABLE IV  
THE EFFECT OF OVERGRINDING TWO COMMERCIAL FLOURS

Sample No.	Protein on moisture-free basis	Maltose per 10 g. of flour in 1 hr. at 30°C.		Starch granules of 20 $\mu$ diameter or over staining with congo red		Maltose produced in 1 hr. at 30°C. by enzyme extracted from 10 g. flour on protein-free basis	
		Original flour	Over-ground flour	Original flour	Over-ground flour	Original flour	Over-ground flour
	%	mg.	mg.	%	%	g.	g.
28	9.7	105	252	10.5	24.3	52.5	49.2
30	10.0	153	282	12.6	28.1	56.1	51.1

In seeking an explanation for the variation between varieties as shown in Tables I and II, one cannot neglect the possibility of there

being a difference in the size distribution of the starch granules in the flours examined. A sedimentation method was used to study the size of the starch granules. Some method of separation of the starch was necessary which would retain all the granules, both large and small. On merely washing out with water some of the small granules are left behind in the gluten. The flour was therefore treated by the method of Hartmann and Hillig (1926) in which the gluten is digested with pepsin. This was carried out in an acid solution to inhibit the amylase action during digestion.

In this separation, 25 g. of ether-extracted flour were digested overnight at 37°C. in 300 ml. of 1% hydrochloric acid containing 1.25 g. of pepsin. The starch was separated by centrifuging, the supernatant liquid discarded, and the starch resuspended in 300 ml. of the acid-pepsin mixture. After the second overnight digestion, the starch was again separated by centrifuging, the supernatant liquid poured off as completely as possible, and the starch suspended in tap water and diluted to 1200 ml. in a tall stoppered cylinder of about 6.5 cm. diameter. The suspension was maintained at 15°C. in a constant-temperature room. After thorough mixing, 20 ml. portions were taken out by pipette and placed in tared porcelain evaporating basins for drying. The depths and the times after mixing at which the samples were taken were so chosen as to cover the progress of the sedimentation to a point when 10% or less of the starch was still in suspension. Beginning with the greatest rate of fall, the first aliquot was withdrawn at 30 cm. depth after 1 minute (by stop watch) after mixing. The suspension was again mixed and the second aliquot taken after 2 minutes at 30 cm. depth. This process of mixing, allowing to stand for the appropriate time, and taking the aliquot at the proper depth was continued for the remaining samples required according to the schedule set out in Table V. The amounts of sediment in the first three aliquots agreed very closely and constituted 100% in suspension,

TABLE V

Aliquot	Time interval after mixing			Depth at which aliquot was taken	Rate of sedimentation
	hr.	min.	sec.		
1	0	1	0	30	0.5000
2	0	2	0	30	0.2500
3	0	4	0	30	0.1250
4	0	6	0	20	0.0555
5	0	7	28	20	0.0446
6	0	15	47	20	0.0211
7	0	22	13	20	0.0150
8	0	47	0	20	0.0070
9	2	13	20	20	0.0025

since down to this rate of fall corresponding to 0.125 cm./sec. no diminution in the concentration of starch granules had occurred at the depths and times specified. The amount of starch in each progressive sample gradually decreased until the termination of the sedimentation measurements corresponding to a rate of fall of 0.0025 cm./sec.

A slight excess of 2% ammonia (about 0.75 ml.) was added to the evaporating basins holding the 20 ml. aliquots before careful drying on a sand bath. After the main portion of the water was removed, drying to constant weight was completed by heating to 110°C. in an electric

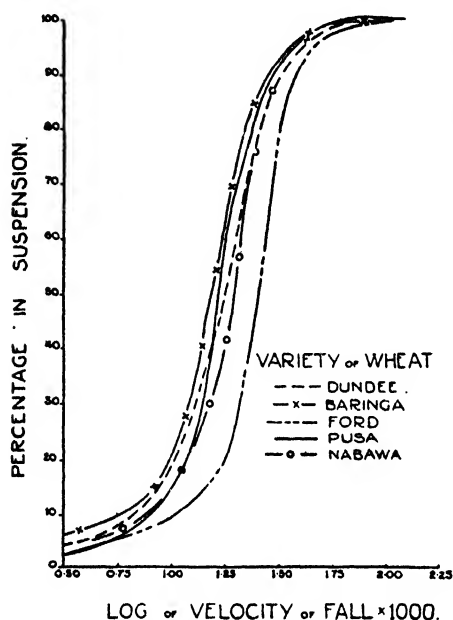


Fig. 3. Summation curves illustrating the sedimentation of starch granules from five varieties of wheat grown in New South Wales, 1936.

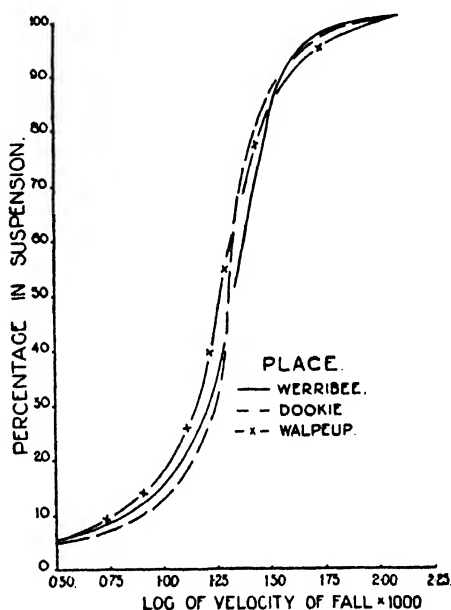


Fig. 4. Summation curves illustrating the sedimentation of starch granules from the same varieties of wheat grown at three places in Victoria, 1936.

oven. The weight of material in each aliquot was corrected for the trace of soluble material present in the sedimenting liquid by drying 20 ml. of the clear supernatant liquid obtained by centrifuging a portion of the suspension. The viscosity and density of this liquid was also determined and found to be practically constant from sample to sample.

Although the density of the starch granules in such a water suspension is not known, the rate of settling as recorded in a sedimentation curve provides a basis for comparisons of the characteristics of the starch granules obtained from different samples of flour. Sedimentation curves typical of those obtained for the starch from six different varieties of wheat grown in Victoria and five grown in New South Wales are shown in Figures 2 and 3. If the flours made from different

varieties of wheat contain different-sized distributions of granules, according to Stoke's law, such a sedimentation curve should reflect that size difference. On the other hand differences in average density of the granules from flour to flour would also be evident in sedimentation curves. The differences observed in Figures 2 and 3 between the curves is such that the time taken for sedimentation of 50% of the starch increases in the order of Ford, Nabawa, Dundee, Pusa 4, and Baringa. This, it will be noted, is in the reverse order to that observed for the extent of injury to the starch granules as shown by staining with congo red. In other words, varieties that suffer extensive damage

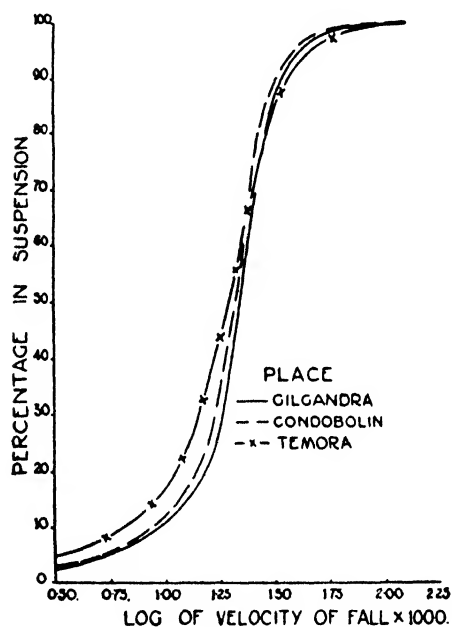


Fig. 5. Summation curves illustrating the sedimentation of starch granules from the same varieties of wheat grown at three places in New South Wales, 1936.

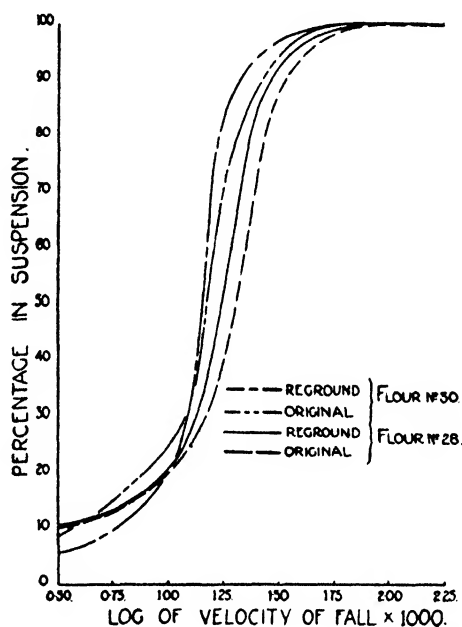


Fig. 6. Summation curves illustrating the effect of overgrinding on the sedimentation of starch granules from two samples of commercial flour.

to their starch granules during milling, settle out of a suspension more slowly than do varieties which show little damage to the granules. This is in accord with what is known regarding the swelling which a starch granule undergoes when it has been injured (Alsberg, 1938). As a result of the swelling, the diameter of the starch granule is of course increased somewhat with a consequent decrease in density.

In contrast with the marked differences noted between the sedimentation curves for the starch granules of different varieties, the influence of locality during the growth of the wheat plant is very slight (Figs. 4 and 5). This is true for both the New South Wales samples and the Victorian samples and may be due to lack of sufficient variation between the soil or climatic conditions of the six places. The examina-

tion of the same varieties from other seasons can give a partial answer to this question. In any case the similarity between the sedimentation curves in Figures 4 and 5 is paralleled by a similarity between the percent of injured granules in the flour samples from the six places.

The effect of increased injury to the starch granules was noted by examination of the sedimentation rate of the starch granules from a given flour which had been subjected to two grinding operations. The original and overground commercial flours Nos. 28 and 30, already described in Table IV, were digested with the acid-pepsin mixture as noted above and then sedimentation curves obtained (Fig. 6). The effect of increased injury to the starch granules is most noticeable when the sedimentation is only partially completed, that is, when the larger granules are being deposited whilst during the later stages of the sedimentation there is little difference between the reground flours and the original flours. The examination of the starch-granule sediment microscopically supports this in that there is no evidence of extensive amounts of starch-granule fragments in the reground flours.

The volume of the sedimented starch granules from the reground flours is greater than from the original flours. The increase in the water-binding capacity of the injured starch granules over that for uninjured granules may have an important bearing on the water-absorption figure for a flour. The moisture contents of the starch sediments from the original and overground samples of flour (No. 30) were compared after concentrating the sediment by centrifuging. All supernatant liquid was poured off and the firmly packed starch was weighed and dried at 110°C. The overground starch had a moisture content which was 8.5% higher than the original starch.

The starch from such a variety as Pusa 4 is similar to that from the overground flour No. 30, in that both contain relatively high proportions of damaged starch granules, while the starch from Ford on the other hand is similar to that from the original flour No. 30, in that both contain a relatively low proportion of damaged starch granules. In order to determine whether the similarity as noted above extends to the water-binding capacity of the starch of the different samples, these two varieties, Pusa 4 and Ford, grown at Werribee were subjected to an acid-peptic digestion. The volume of the starch from 5 g. of Pusa 4 was 9.6% greater than that from the same amount of Ford and the moisture content of the Pusa 4 starch was 12.3% higher than for the Ford starch.

Since the sedimentation method of studying the size of starch granules gave more information on the treatment to which they had been subjected in milling than it did on the actual size of the original granules, direct measurements were made. The starch granules for these

measurements were prepared by digesting 5 g. of flour with a solution of pepsin overnight, mixing the suspension well and placing small drops on a clean slide. The drop was diluted with water and spread over an area of about 2 sq. cm. and allowed to dry for three days in the air. For examination, an anhydrous liquid, *e.g.* lactic acid, was used to prevent swelling of the granules. Measurements were made at 500 $\times$  using a projection microscope and a glass mm. ruler. A minimum of 500 starch granules of 8 $\mu$  or over in diameter were measured in each sample. The average diameter was taken as the arithmetic mean of the long and short diameters of the starch granule.

Data were collected on the size distribution of starch granules in 16 samples of flour from 4 different varieties of wheat. A volume distribution curve was calculated on the basis of the measured diameters. Starch granules below 8 $\mu$  in diameter were not counted for all the samples, so that in order that all the results be comparable, only granules of 8 $\mu$  diameter or over were included in the calculation of the volume distribution for the different samples. From the data available (Dadswell and Wragge, 1937) these small granules make up about 95% of the total number of granules so that it is evident that the frequency curve for percentage of total number in relation to diameter is very much asymmetrical. However, in spite of their overwhelming numbers, the small granules constitute only 7.5% of the total volume of the starch.

Those summation volume-distribution curves in Figures 7 and 8 represent the maximum variation due to variety and place of growth for the 16 samples studied. This variation is not of the magnitude of the difference between the sedimentation curves in Figures 2 and 3.

Varietal differences among the samples examined were of about the same magnitude as the differences due to place of growth.

The protein contents of the samples as set out in Table VI bear no significant relation to the proportion of injured granules when all the samples are considered regardless of source or variety (correlation,

TABLE VI  
THE PROTEIN CONTENT ON A MOISTURE-FREE BASIS

Place	Baringa	Pusa 4	Nabawa	Ford	Dundee	Free Gallipoli
	%	%	%	%	%	%
Dookie, Vic.	10.4	13.5	9.8	11.4	—	9.6
Werribee, Vic.	14.2	12.3	10.4	13.5	—	13.4
Walpeup, Vic.	12.3	13.4	12.3	—	—	15.5
Temora, N. S. W.	10.9	14.7	11.1	11.6	11.8	—
Gilgandra, N. S. W.	13.6	13.4	11.8	14.8	14.1	—
Condobolin, N. S. W.	14.5	14.9	15.0	11.6	13.0	—

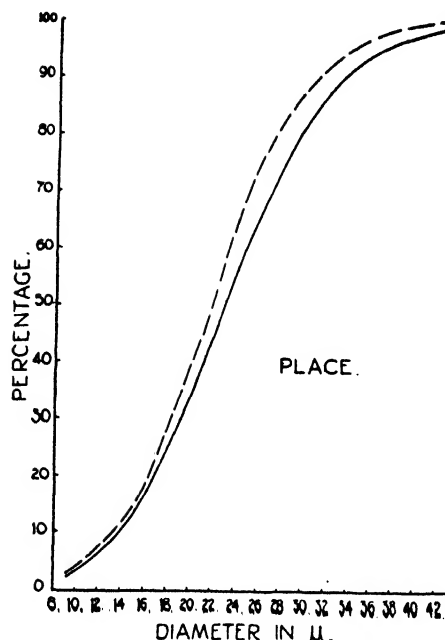


Fig. 7. Summation curves showing the maximum difference in volume distribution of starch granules in relation to diameter, as determined by direct measurements, for the same varieties of wheat grown at two places in Victoria.

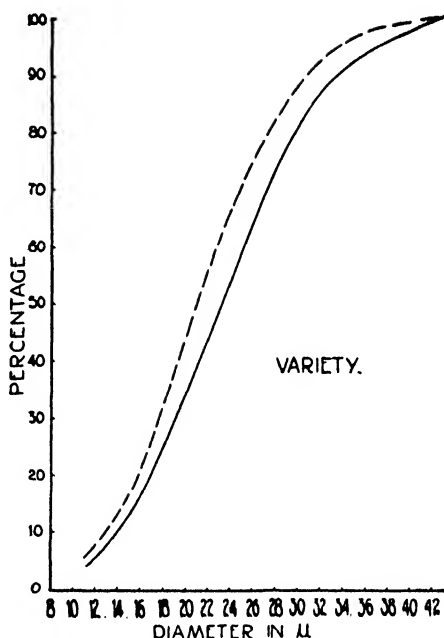


Fig. 8. Summation curves showing the maximum difference in volume distribution of starch granules in relation to diameter, as determined by direct measurements, for four different varieties of wheat grown at the same places in Victoria.

-.091). But when the samples of one given variety are considered there is a tendency for those having the highest protein content to have the fewest granules capable of staining with congo red and the lowest maltose figure. The work carried out by Breakwell and Hutton (1939) in South Australia on the relation between protein content and maltose figure indicates that whole meal from areas producing high-protein wheat has a lower maltose figure than whole meal from wheat grown in areas producing low-protein wheat. Graesser (1936) found that there was a significant negative correlation between the diastatic activity and protein content of 25 randomly chosen second-patent and straight-run Canadian Western hard-wheat flours. On the other hand work by Geddes and Aitken (1935) on flour made from 28 samples of wheat from 11 different countries indicates no correlation between diastatic activity and protein content.

It is possible that the higher protein content of some samples acts in such a way as to shield the starch granules from injury in milling, although such injury as noted in the samples reported on in this paper is more closely related to variety than to the protein content.

## II—Enzyme Activity

It is believed from the evidence presented by other workers (see discussion in introduction) that beta-amylase is the most active amylase

present in normal flour milled from sound wheat, and that it is the one which is largely concerned in the formation of maltose in an autolytic digestion. Consequently a very dilute cold-water extract of the various flours studied and reported on in this paper was made and allowed to act on boiled soluble starch. It is considered that this procedure is justifiable as a measure of the enzyme concentration where the rate of maltose formation is dependent on the concentration of enzyme, as in the case of beta-amylase and the raw starch factor, *i.e.*, when little or no alpha-amylase is present.

Beta-amylase in the actual autolytic digestion of flour is considered to be acting on that portion of the starch substrate which is comparable to the starch ground in a rod mill or to boiled starch (Stamberg and Bailey, 1939b); therefore the latter was used as the substrate for assessing the enzyme activity. In order that a lack of the substrate would not be a limiting factor in the assessment of the concentration of enzymes, a large excess of the buffered boiled starch was used.

### Experimental

Five grams of flour were mixed with 100 ml. of cold water and allowed to stand one-half hour with frequent shakings. One ml. of filtrate of the flour suspension was allowed to act at 30°C. on 157.5 ml. of 2% soluble starch which had been mixed with 14 ml. of water and 57.5 ml. of buffer solution which contained 12 ml. of glacial acetic acid and 16.4 g. of anhydrous sodium acetate per litre. The soluble starch was made up according to the directions given by the American Association of Cereal Chemists (1935). A blank was run on the mixture after the flour extract had been added. At the end of one hour, during which the flasks were shaken at 15-minute intervals, another sample was taken for a maltose estimation by the Blish-Sandstedt method (American Association of Cereal Chemists, 1935). The results were calculated as grams of maltose produced by the amylase in the water extract from 10 g. of flour.

An indication of the part which the activity of the enzyme may play in determining the maltose figure in the autolytic digestion of flour may be judged from observing the values for amylase strength as set out in Table VII.

The activity of the enzyme extracts varies from flour to flour and bears little consistent relation to variety except for the samples of Free Gallipoli which have a marked tendency toward the highest activity. The samples from Victoria have, in general, a higher amylase activity than do those from New South Wales, and likewise the maltose figures obtained by autolytic digestion (Table II) are slightly higher for the Victorian samples. Considering the individual samples the



TABLE VII

THE AMYLASE STRENGTH—AS MALTOSE PRODUCED BY THE ACTION OF A WATER EXTRACT FROM 10 G. OF FLOUR ON A PROTEIN-FREE BASIS, ACTING ON 2% SOLUBLE STARCH FOR 1 HOUR AT 30°C.

Place	Baringa	Pusa 4	Nabawa	Ford	Free Gallipoli
	g.	g.	g.	g.	g.
Dookie, Vic.	52.4	87.9	46.4	67.6	59.4
Werribee, Vic.	65.0	81.5	48.8	43.9	106.5
Walpeup, Vic.	68.0	80.2	59.8	—	104.4
Temora, N. S. W.	45.1	15.4	53.6	16.6	—
Gilgandra, N. S. W.	37.8	56.8	7.9	53.5	—
Condobolin, N. S. W.	25.9	—	26.9	10.4	—

over-all correlation between amylolytic activity and the maltose figure by autolytic digestion is  $+0.206$ , which is not significant.

The amylolytic activity of the samples bears no relation to their protein content. Severe overgrinding reduces the amylolytic activity of the two samples of flour considered in Table IV.

### Summary

Six representative varieties of wheat grown in Victoria and New South Wales have been studied.

Injury to starch granules in milling is correlated with variety but not with place of growth.

The maltose formed during the autolytic digestion of flour is positively correlated with the extent of injury to the starch granules and consequently with variety.

Two samples of commercial flour were subjected to overgrinding with a consequent increase in both the maltose figure and the proportion of injured starch granules.

An increase in the proportion of injured starch granules is accompanied by an increase in their wet volume and a higher water-binding capacity.

Measurements of the average diameter of starch granules from four different varieties grown at four places indicate that the variation in the volume distribution of the granules due to environment is of approximately the same magnitude as that due to variety.

The protein content of the flours examined bears no correlation to the proportion of injured starch granules or to the maltose figure. Within a variety however there is a slight tendency for the highest protein content to be accompanied by the lowest injury to the starch granules.

The amylolytic activity of the water extract from the flours examined varies widely and bears no significant relation to the maltose figure.

### Conclusions

The results of this work indicate that different varieties of wheat require entirely different treatments by the miller in order to obtain a similar degree of availability of the starchy portion of the flour, but it is probably more feasible for them to blend the wheat of different varieties carefully in order to standardize the gassing power of flours.

A still more fruitful field would seem to be that of selection for growth of those varieties of wheat having the desired inherent properties not only as has been done in regard to protein but also on the basis of availability of the starch for enhancing the gassing power of the flour.

### Acknowledgments

The authors wish to thank Mr. W. R. Jewell, Agricultural Research Chemist, Department of Agriculture of Victoria, and Mr. L. S. Cayzer, Chemist, Department of Agriculture of New South Wales, for providing the flour samples used in this work. The encouragement and interest shown by Professors W. J. Young and S. M. Wadham, of the University of Melbourne, has also been very much appreciated. The advice and help rendered in the statistical analysis of the data by Dr. H. C. Forster of the Department of Agriculture of Victoria, is gratefully acknowledged.

The University of Melbourne has been very generous in providing the Research Scholarships held by the authors during the time that the work reported on in this paper was carried out.

### Literature Cited

- Alsberg, C. L.  
1927 Starch in flour. *Cereal Chem.* 4: 485-492.
- Alsberg, C. L.  
1938 The structure of the starch granule. *Plant Phys.* 13: 295-323.
- American Association of Cereal Chemists  
1935a Cereal laboratory methods (3rd ed.). Published by A.A.C.C.
- Andrews, J. S., and Bailey, C. H.  
1934 The amylases of normal and germinated wheat flours. *Cereal Chem.* 11: 551-556.
- Bailey, C. H.  
1925 The chemistry of wheat flour. Published by Chemical Catalog Co. Inc., N. Y.
- Blish, M. J., Sandstedt, R. M., and Kneen, E.  
1938 The cereal amylases with reference to flour and malt behavior. *Cereal Chem.* 15: 629-657.
- , —, and Mechem, D. K.  
1937 The action of wheat amylases on raw wheat starch. *Cereal Chem.* 14: 605-628.
- Bottomley, R. A.  
1938 The relationship between diastatic activity (maltose figure) and "gassing power" of experimentally milled flours from some Australian wheats. *Cereal Chem.* 15: 509-520.
- Breakwell, E. J.  
1938 Studies on the effect of weathering on the commercial value of wheat samples from the 1937-8 harvest. *J. Agr. S. Australia* 41: 777-790.
- and Hutton E. M.  
1939 South Australian grain studies. *J. Agr. S. Australia* 42: 683-697.
- Brown, H. T. and Heron, J.  
1879 Contributions to the history of starch and its transformation. *J. Chem. Soc.* 35: 596-654.
- Collatz, F. A., and Racke, O. C.  
1925 The effects of diastase and malt extract in dough. *Cereal Chem.* 2: 213-227.

- Cutler, G. H., and Brinson, G. A.  
1935 The granulation of whole wheat meal and a method of expressing it numerically. *Cereal Chem.* **12**: 120-129.
- Dadswell, I. W., and Wragge, W. B.  
1937 Unpublished data.
- Eva, W. J., Geddes, W. F., and Frisell, B.  
1937 A comparative study of various methods of measuring flour gassing power. *Cereal Chem.* **14**: 458-480.
- Farquhar, A. J.  
1938 The baking quality of bleached and sprouted wheats. *J. Agr. S. Australia* **41**: 649-651; 776.
- Geddes, W. F., and Aitken, T. R.  
1935 An experimental milling and baking technique requiring 100 grams of wheat. *Cereal Chem.* **12**: 696-707.
- Graesser, F. R.  
1936 Correlation between volume of loaf, crumb characters, baking value, and diastatic activity in Canadian Western hard wheat flours. *Cereal Chem.* **13**: 356-363.
- Gründer, W.  
1935 Der Einfluss der Korngrösse bzw. freien Oberfläche auf Kleber- und Teigcharacteristik bei Weizenmehlen. *Mühlenlab.* **5**: 17-26.
- Hanes, C. S.  
1937 The action of amylases in relation to the structure of starch and its metabolism in the plant. Part I. *The New Phytologist* **36**: 101-140, 189-239.
- Harris, R. H., and White, S. N.  
1938 A study of various characteristics of mill-stream flours and their relation to loaf volume. *Cereal Chem.* **15**: 489-500.
- Hartmann, B. G., and Hillig, F.  
1926 The influence of peptic digestion in the determination of total carbohydrates in cereal products. *J. Assoc. Off. Agr. Chem.* **9**: 482-484.
- Hermano, A. J., and Rask, O. S.  
1926 A consideration of certain reactions of starches with special reference to enzyme hydrolysis. *Cereal Chem.* **3**: 361-392.
- Hickinbotham, A. R.  
1936a Tests of quality adapted to hand-ground meals. *J. Agr. S. Australia* **40**: 135-140.  
1936b *Ibid.* **40**: 277-289.
- Hills, C. H., and Bailey, C. H.  
1938 The amylase of different barley varieties. *Cereal Chem.* **15**: 351-362.
- Jewell, W. R.  
1935 Our daily bread. *J. Agr. Victoria* **33**: 331-339.
- Johnson, A. H.  
1930 Report on diastatic value of flour. *J. Assoc. Off. Agr. Chem.* **13**: 445-446.
- Karacsonyi, L. P., and Bailey, C. H.  
1930 The relation of overgrinding of flour to dough fermentation. *Cereal Chem.* **7**: 571-587.
- Kent-Jones, D. W.  
1927 *Modern cereal chemistry* (2nd ed.).
- Leatherock, L. E., McGhee, H. W., and Giertz, J. W.  
1937 Diastatic activity in connection with flour mill control. *Cereal Chem.* **14**: 161-181.
- Malloch, J. G.  
1929 Studies on the resistance of wheat starch to diastatic action. *Canad. J. Res.* **1**: 111-147.
- Mangels, C. E.  
1926 Factors influencing the diastatic activity of wheat flour. *Cereal Chem.* **3**: 316-322.  
1934 Varietal and regional variations in properties of wheat starches. *Cereal Chem.* **11**: 571-585.  
1936 Varietal and regional variations in durum wheat starches. *Cereal Chem.* **13**: 221-233.
- Markley, M. C., and Bailey, C. H.  
1934 Factors affecting the diastatic activity of wheat flour. *Cereal Chem.* **11**: 515-522.

- Pascoe, T. A., Gortner, R. A., and Sherwood, R. C.  
1930 Some comparisons between commercially and experimentally milled flours. *Cereal Chem.* 7: 195-221.
- Pulkki, L. H.  
1938 Particle size in relation to flour characteristics and starch cells of wheat. *Cereal Chem.* 15: 749-765.
- Sandstedt, R. M.  
1938 The relation of alpha- and beta-amylases to the evaluation of malts in flour technology. *Cereal Chem.* 15: 832-835.
- , Blish, M. J., Mecham, D. K., and Bode, C. E.  
1937 The identification and measurement of factors governing diastasis in wheat flour. *Cereal Chem.* 14: 17-34.
- Stamberg, O. E., and Bailey, C. H.  
1939a The effect of adding alpha- and beta-amylases to dough. *Cereal Chem.* 16: 42-50.  
1939b Studies on wheat starch. II. The action of amylases on raw wheat starches. *Cereal Chem.* 16: 319-330.
- Swanson, C. O.  
1935 Some factors which affect diastatic activity in wheat. *Cereal Chem.* 12: 89-107.  
1936 Effect of harvest conditions on a few quality factors in wheat. *Cereal Chem.* 13: 79-90.
- West, H. E.  
1932 Diastatic activity of New Zealand wheat: effect of variety and locality. *Wheat Res. Inst. Bul.* 35, N. Z. Dept. Sci. Ind. Res. pp. 40-46.

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## COMPRESSIBILITY OF BREAD CRUMB

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(Read at the Annual Meeting, May 1940)

Much good chemistry and physics has been expended on the physical properties of bread dough. An excellent summary of all this work has been made by C. H. Bailey (1940). Halton and Scott Blair (1937) have gone so far as to measure the viscosity and modulus of elasticity in absolute units. For dough the importance of this is evident. In fermentation, rounding, molding, proofing, and spring in the oven, dough must be capable of deformation without tearing, so that its viscosity and elasticity are among its essential qualities and its suitability for various uses depends directly upon these two qualities and upon the relation between them.

This is equally true in the larger field of biscuit, cracker, and cake doughs. In 1912 the senior author was employed as dough mixer by a large manufacturer of biscuits and crackers. Here the range extends all the way from the completely liquid suspensions of flour used for sugar-wafer batter to the hard-tack doughs that seem to resemble rhinoceros hide more closely than any other common material. The resulting baked products are very sensitive to variations in viscosity and

elasticity of the doughs. He early realized that experienced practical dough mixers perceived qualities in a dough which they did not have the ability to describe clearly in words. As early as 1913 his notes show a conscious effort to express in definite physical constants the desirable characteristics of each dough. Even at that date, before the illuminating work of Bingham on plasticity, his notes show a realization of ductility, elasticity, and stickiness as more or less distinct physical characteristics to be considered. Like so many others of that date he was groping for the ideas now so clearly expressed by Scott Blair (1939).

For the finished baked product the importance of physical measurements is not so self-evident as it is for the dough. Bread and biscuit doughs may have to flow and stretch, but the finished bread and biscuits do not have to do so to any considerable extent. Hence physical characteristics are worthy of study only to the extent that their importance can be demonstrated. Unlike the measurement of physical properties of doughs, such measurements on baked products carry the obligation to show that such measurements are worth making and have some real meaning.

Measurements of the compressibility of bread crumb seem to be justified for the following reasons:

1. Progress in general is stimulated by the development of reliable, quantitative methods. Especially in the food industries there is a general need for objective measurements to replace mere opinions with their well known shortcomings.

2. Softness in bread is recognized as a desirable characteristic. Within certain limits and other things being equal, the softer the bread the better. Therefore a method for measuring this quality conveniently is worth while.

3. Softness or compressibility is recognized as an important factor in freshness. As a result of a prolonged consumer preference study, Stateler (1936), reporting for the New York Section of the American Association of Cereal Chemists, said: "From all these tests only one factor seems to have any special significance. That factor was freshness. In bringing out that factor the experts as well as the consumer group were more nearly consistent in their conclusions." It has been shown that measurement of rate of change in compressibility is one of the best methods for following the loss of freshness or rate of staling.

4. Compressibility is shown to have diagnostic value as an indication of the approach to the optimum in several factors such as mixing and fermentation.

5. Compressibility of crumb doubtless has many points of purely theoretical scientific interest, but these are not explored in the present report.

### Previous Work

Measurements of the breaking and crushing strength of biscuits and crackers were made by Davis (1921) and by Platt and Fleming (1923), and the method described was also used to measure the shortening power of fats and the softness of flours. Physical measurements on cakes were described by Platt and Kratz (1933). The softness of bread crumb was measured by Katz (1917) and his method summarized in English by Katz (1928). He used the cut surface of a loaf without removal of crust. Katz used this method to measure rate of staling. Further work along these lines was carried out by Platt (1930) who gives the details of a simple apparatus made by attaching a plunger to the bottom of one pan of a large balance. Platt used a slice of bread  $2\frac{1}{2} \times 2\frac{1}{2}$  inches and  $1\frac{1}{2}$  inches thick with the crust trimmed off. He describes a convenient method for carrying out this test and gives the effects of temperature, age, and varying weights on the compressibility of bread crumb. L. H. Bailey (1930, 1932) describes a simple, though not very sensitive, apparatus for the same purpose. A prism of bread crumb  $1\frac{1}{2} \times 1\frac{1}{2} \times 2$  inches is subjected to a load of 500 or 1000 g. Change in compressibility measures rate of staling.

Steller and Bailey (1938) studied the effects of various factors on the staling of bread using viscosity, sedimentation, and compressibility tests. For the latter they used an apparatus similar to that of Platt. Staleness was expressed as the percentage loss of compressibility as compared with the compressibility of bread one hour out of the oven as the standard. This gave quite sensitive and concordant results. A possible objection to this method is that no data are given as actually observed. All are stated as a percentage of the findings on the bread when one hour old. Unfortunately determinations made on very fresh bread have the greatest experimental errors, due to difficulties in slicing fresh bread, difficulties in temperature control, and to the fact that at this age the condition of the bread is changing very rapidly, as can be seen from any staling curve. These errors could probably have been reduced by selecting an age of two, three, or four hours (or an average at two of these ages) to form the base from which other data would be calculated on a percentage-of-loss basis.

Two important conclusions from this paper are: (1) "Flour strength appears to be an important factor in the aging of bread crumb, the rate of staling being a function, though not linear, of the protein content of the flour." (2) "Compressibility and viscosity measurements of staleness were more consistent and uniform than the data obtained by the sedimentation method."

An interesting departure from the usual methods for measuring the

compressibility of bread was devised by Morison and Coriolis (1929). The rectangular prism of bread crumb to be tested is  $2\frac{1}{2} \times 2\frac{1}{2} \times 3$  inches. This is subjected to a 7-pound (3175 g.) weight for 15 seconds and the position of the plunger noted. The weight is then removed and the bread is allowed to spring back towards its normal shape for 30 seconds. The percentage of rebound is calculated. In this case the weight is many times that used by any other investigator. The effect is made the more severe by the fact that the weight is applied suddenly, giving considerable impact before its fall is arrested. This instrument was called a "penetrometer."

The foregoing test was originally devised to determine when a loaf was sufficiently baked. It is the only objective test for this quality known to the present authors. Interesting effects on the readings with this penetrometer were also shown by the amount of fermentation, richness of formula, strength of flour, and other factors. Cathcart and Pushnik (1939) state: "A loaf that is gummy or one that is unbaked will show a low per cent of rebound. One that is well baked and seems to chew well or have good eating qualities will have a higher rebound. All these tests were performed on bread at the time of scoring. Penetrometer readings have been correlated fairly well with the score for 'Bread Quality.'" Here we have a definite statement of the connection between compressibility results and eating quality.

The object of the present paper is threefold: first, to arrive at a clearer understanding of what happens in making a compressibility test; second, to make available a number of recent improvements and refinements in methods for making compressibility measurements; third, to present the results of such measurements as affected by various factors and to point out their practical application.

### Details of the Improved Method

The basic method of making the compressibility test is briefly as follows: The apparatus as depicted in Figure 1 consists of large balance having a plunger fastened to the under side of one of the pans. The piece of bread to be tested is placed on a platform immediately below the plunger with the plunger resting on the upper surface of the bread. The weight is then applied for a specified time and the amount of compression measured by means of a pointer which magnifies the movement 3.9 times. This simple instrument has been described and illustrated by Platt (1930).

Changes in details of the new method from the method described in 1930 are as follows: The face of the plunger has been enlarged from 31 mm. diameter to 36 mm., the latter having a surface of 10.2 sq. cm.

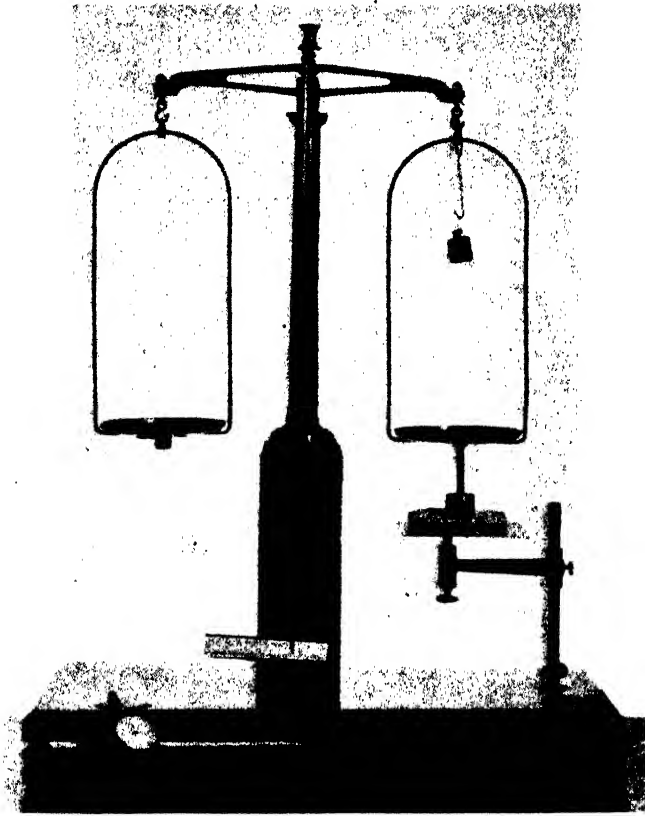


Fig. 1. Balance type of apparatus for measuring compressibility. Note plunger resting on slice of bread being tested.

The larger face is found to decrease the error because it is less affected by small local irregularities in grain and texture of the bread surface. The time of action has been reduced to 15 seconds.

The graphs of compressibility vs. time are shown in Figures 2 and 3. Evidently most of the compression takes place in the first few seconds. About 67% has occurred in the first 5 seconds and 72% in 15 seconds. After 15 seconds the pointer indicating the downward movement of the plunger on the bread is moving very slowly. The percentage error in timing and reading is negligible. There is no indication of a definite end point; neither is there any evidence that anything is gained by continuing the load for much longer periods. On the other hand there are definite advantages in a short period for each test, so that many tests can be made on bread at approximately the same age. Because of variations between slices and between loaves a rather large number of tests is necessary in order to get a broad view of the variation



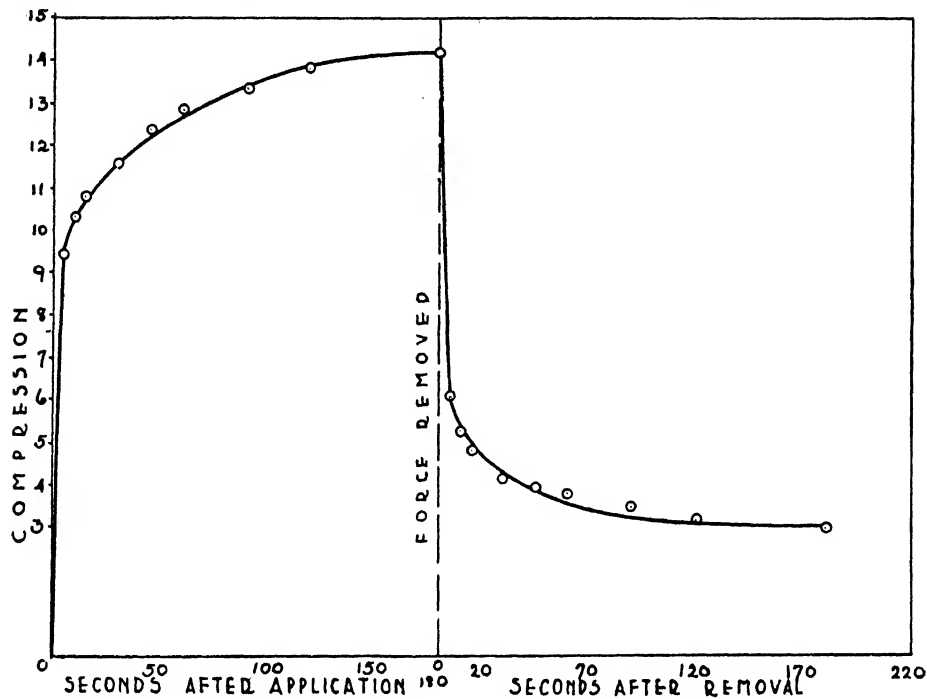


Fig. 2. Effect of time on compression after addition and removal of the force. Weight 100 g. Bread 24 hours old. Each point is the average of four slices from the same loaf.

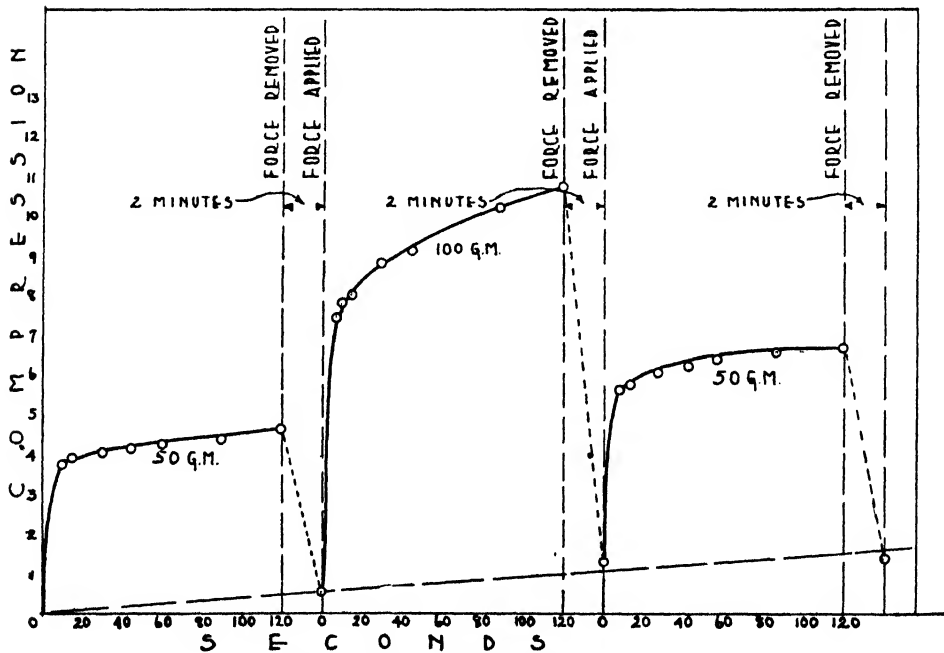


Fig. 3. Effect of repeated compression.

of compressibility in the batches under consideration and in order to increase the significance of the results.

The addition of a weight direct to the balance pan over the plunger which compresses the bread is found to introduce errors due to the jarring effect of the sudden application of the weight. One satisfactory method for avoiding this is by the use of a chain as a counterweight. The chain is removed from the left pan instead of adding a weight to the right pan (Platt, 1930). This is an entirely satisfactory method when the time of application of the stress is considerable. However, for a 15-second total application a quicker method is desirable. A quick addition of the weight without the production of a disturbing impact was accomplished by adding the weight to the righthand side of the balance by hanging it on a hook which was in turn suspended by a rubber band. The stretching of the rubber absorbs the jar, which if present causes a measurable effect on the readings.

Adjustment to zero and the final reading of the pointer are made with the aid of a large lens. The end of the pointer has been sharpened so that readings are made by estimating to tenths of a scale division. Each scale division measured by the pointer corresponds to  $1/3.9$  or about 0.26 mm. descent of the plunger in compressing the bread.

While compressibility tests on bread at all ages from one hour to 72 hours are of interest, the tests of most practical interest are those between 12 and 48 hours old, as this is the age at which most bread is actually eaten. Errors are also less than those made on very fresh bread.

In all the previous work on the compressibility of bread crumb already cited the tests have been made on rather thick pieces of bread usually cut as rectangular prisms with the crust trimmed off. Relatively large stresses with correspondingly large resulting strains were used. A useful improvement has consisted in making the compressibility tests on ordinary slices of commercial thickness (about  $\frac{1}{2}$  inch) without removal of the crust. The possibility of doing this was first pointed out by Baker (1939).

Testing ordinary slices has the following advantages over testing specially cut thick rectangular prisms of bread crumb: (1) Convenience. (2) Saving of time. (3) Great increase in the number of replicates per loaf, thereby reducing the error of the mean. (4) Considerable reduction in the error of slicing. The best commercial bread slicers cut a remarkably uniform slice with no apparent injury to the crumb from crushing or tearing. However, when cutting a piece of bread of special size and shape for testing, a considerable error may be introduced in the cutting, and special skill is necessary. (5) Bread is eaten by the slice,

so that there is real meaning in studying the slices themselves as is discussed below.

When testing laboratory loaves made in a pan of commercial size and shape, best results are obtained by slicing them for testing on a commercial slicer. However, if such is not available excellent results can be obtained by the use of a mitre box. A convenient form is shown in Figure 4. The base is made of hard wood having the same width

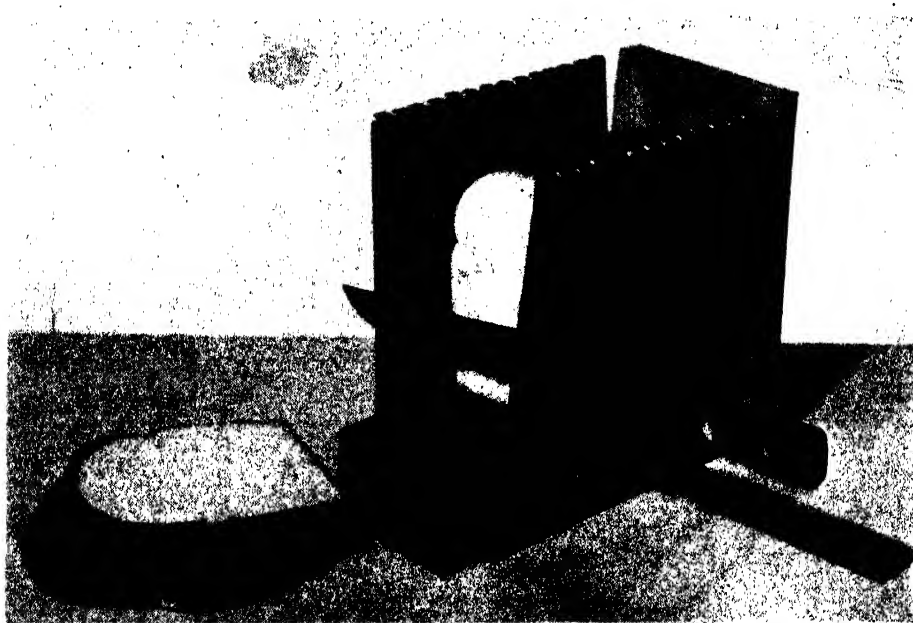


Fig. 4. Mitre box for precision slicing of laboratory loaves.

as the pan in which the bread was baked. Guides are provided by brass strips 4.5 mm. thick and 12 mm. wide. The strips are placed 1 mm. apart to allow the insertion of the bread knife. These strips are placed at the same angle from the vertical as the sides of the bread pan in which the loaf was baked. A Burns bread knife with saw-tooth edges does the best job in cutting the bread. Such a mitre box exactly fits the loaf and supports it while it is being cut. The box produces slices with parallel surfaces and of uniform thickness.

. Bread purchased in the grocery store which has been commercially sliced and wrapped often contains slices which are somewhat warped so that they do not lie flat. Many are "cupped" so that their two surfaces are concave and convex respectively. If such a slice is placed under the plunger with the concave side downward and the weight is then applied, results much too high will be obtained. This is because the plunger must first travel some distance in flattening out the slice

and in pushing the lower surface of the slice firmly down against the supporting platform before actual compression of the bread occurs. This error may be avoided by turning the slice so that it is concave upward, in which case the lower surface under the plunger may be caused to lie flat against the supporting platform.

Another method which is even more satisfactory is to place a metal disk about 1.5 mm. thick and 38 mm. in diameter on the platform supporting the slice of bread directly under the plunger. Such a disk supports the lower surface of the slice so as to prevent its bending downward under the plunger when cupped. In all slices whether cupped or not this disk tends to localize the effect of the stress directly under the plunger and lessens the spreading of the effect of this stress to remoter parts of the slice.

One result of measuring compressibility on slices of bread of the usual size was to bring out the differences in the compressibilities of different parts of the same slice—a point first brought to the writer's attention by J. C. Baker. These variations are of three kinds: first, random differences due to lumps, holes, and other accidental small local variations in texture—most of them quite evident to the eye or finger; second, the expected loss in compressibility due to a too close approach to the crust; and third, more interesting than these, the consistent difference found in most normal slices of bread. Twist bread introduces additional irregularities not considered here.

It might be expected that the geometrical center or the point farthest removed from the crust would be the softest, but such is seldom the case. In pan bread the softest part is usually in the center about one third of the way from the bottom of the slice. In 27 slices taken from 3 loaves, all about 20 hours old, measurements were made in succession on the upper third, the center, and the lowest third of the slice. Results at two different ages are shown in Figure 5. At 20 hours the lowest third is found to be 1.9 times as compressible as the upper third. At 50 hours the factor was 1.3 in this case. This distinct difference in the compressibility of different parts of the same slice makes it important that when differences in compressibility are being considered, the same place on each slice should be measured in every case.

When a slice is under the plunger for test the area of the slice which is being compressed can be marked by touching the surface of the bread at the edge of the plunger nearest the observer with an inked pen. This can be done while the test is being made without affecting the results. The small ink spot makes it possible to apply the plunger to the same location if a second test is required.

In previous studies of rate of staling it has always been necessary to study one loaf or group of loaves to establish data for the fresh bread,

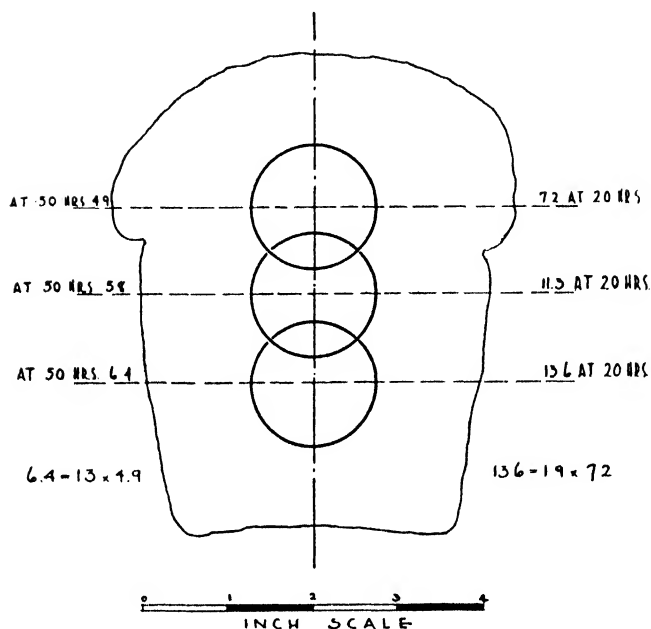


Fig. 5. The lowest third of a slice is usually the softest. Figures represent the average compression of all slices from three loaves, each slice tested at the three points shown.

and then a different loaf or set of loaves to establish the data for the bread at each succeeding age. This method really measured the compressibility of one group of loaves when fresh and of another group out of the same batch when stale. Thus the accidental differences between loaves were included in the experimental error, decreasing the significance of the difference between fresh and stale readings accordingly.

By testing slices, and preserving these same slices without physical damage in their original order and without loss of moisture, it is possible to test the same loaves and the same slices and the same spot in each slice at two or more ages. Such a method evidently eliminates many errors and improves the reliability of the results.

A convenient way to store slices for these tests is in a bread pan of the same size and shape as the one in which the loaf was baked. The pans are stored in tin boxes with a tight (but not completely air tight) cover. All storage and tests are carried out in an air-conditioned bakery where a temperature of  $78^{\circ}$  and a relative humidity approximately 40% are maintained. The bread is out of the boxes only long enough to make a test and the surface of each slice is exposed only long enough to make the test on that slice and the next. As the testing of the slices proceeds the slices are repiled in the same order. Loaves are of course numbered and any individual slice can be relocated if desired because its numerical order has been preserved.

Contrary to expectations the slices in the center of the loaf are not found to be softer than those nearer the ends. Even when the two slices nearest each end are excluded, the slices toward the end tend to be the most irregular in compressibility and are usually softer (Tables I and II). Cupping, if present, is also more apt to be found in com-

TABLE I  
VARIATIONS FROM SLICE TO SLICE IN SAME LOAF, BETWEEN  
DUPLICATE LOAVES, AND BETWEEN FORMULAS

Water		Compression 4% powdered skim milk	
Loaf A	Loaf B	Loaf P	Loaf Q
11.2	13.0	15.5	14.9
9.7	11.8	11.9	14.0
9.5	9.3	11.9	11.0
8.1	7.9	10.3	12.1
8.0	8.2	13.6	11.0
8.9	9.9	13.0	13.0
9.8	10.6	14.9	14.4
10.0	12.1	17.0	17.2
12.0	13.7	11.9	16.1
AVERAGE EACH LOAF			
9.7	10.7	13.3	13.7
AVERAGE OF DUPLICATES			
10.2		13.5	

TABLE II  
REPRODUCIBILITY OF RESULTS OF RETESTING SAME SLICES IN THE SAME PLACE

1st test	Compression 2nd test	Difference
9.0	9.0	0.0
13.0	13.0	0.0
12.1	12.0	0.1
10.5	10.9	0.4
11.1	11.0	0.1
11.9	11.8	0.1
10.5	10.0	0.5
9.2	9.0	0.2
9.8	9.7	0.1
8.4	8.5	0.1
8.3	8.0	0.3
7.9	7.2	0.7
9.0	9.0	0.0
11.0	10.7	0.3
AVERAGE		
10.1	9.99	.2

Average difference between tests is  $0.2/10 = 2\%$  of mean.

mercially sliced and wrapped loaves in the slices near the end. For this reason we have excluded the two slices nearest each end in short loaves, and the three slices nearest each end in long loaves. One possible reason for the softer slices near the ends may lie in molding conditions. In general the dough near the ends receives less crowding and is less tightly molded than that at the center. The principal reason for recognition of these variations in compressibility is to permit the avoidance of errors which arise when these discrepancies are not taken into consideration.

If we make a chemical test on two samples of a solution taken from the same bottle and find widely varying results, we rightly conclude that our testing method is unsatisfactory. However, if we find wide variations in the compressibility measurements of different slices from the same loaf, we cannot at first be sure whether this indicates a real variation in the slices, or a considerable error in our method, or both. The best plan found for checking the error of the method and thus for selecting the method having the least error, is to test every slice in one or more loaves and then to retest immediately the same slices at the same marked spots. The average of the differences between the tests on the same slice is an indication of the precision of the method.

When large forces are allowed to act for relatively long times and when the weights are applied without care in the elimination of impact the crumb of the bread may be permanently changed by the first test so that the second will show an increase in average compressibility. However, when the test is carried out as described above there is practically no destruction of crumb structure. The second test is found to average 1.05 times the first one. This correction can be made if desired, but is usually negligible. The variation to be expected under favorable conditions between duplicate tests on the same slices is shown in Table II. The variations to be expected from slice to slice, from loaf to loaf, and from batch to batch when the batches are made with different formulas are shown in Table I.

### **The J. C. Baker Compressibility Apparatus**

An entirely different form of apparatus for measuring compressibility has been designed by J. C. Baker (1939). The apparatus is shown in Figure 6. The slice of bread, placed as shown, is acted on by a plunger *A*. Adjustment is made for the thickness of the slice by means of the nut *B*. Scale *C* reads slice thickness direct in millimeters. When the plunger is depressed, the amount of the depression is multiplied 10 times and is read direct in millimeters on scale *D*. The depression is caused by the rotation of a small drum *E*, which is actuated

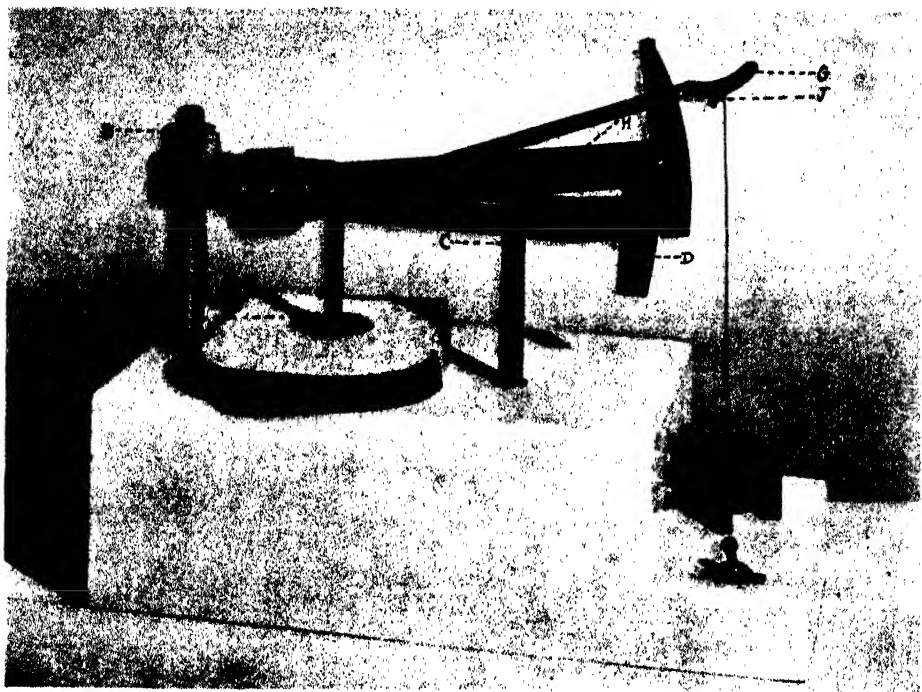


Fig. 6. Apparatus of J. C. Baker for the measuring of compressibility of bread. Stress and strain are indicated simultaneously on scales *J* and *D* respectively.

slowly and uniformly by a motor and shaft located below the bed of the apparatus. The string *F*, which is wound on the drum, serves to depress lever *G*. This lever is connected to lever *H* through a coil spring (not shown because located behind scale *J*).

When the apparatus is in use the amount of the stress at any moment is given on scale *J* and the amount of the corresponding strain or depression of the plunger at the same moment is indicated on scale *D*. The drum *E* is made so that it will slip on the shaft. By this means the drum may be adjusted, wound, unwound, or stopped with the fingers, regardless of the motion of the shaft. By turning the drum manually in this way, either stress or strain may be held constant over a period of time and the resulting change in the other variable noted. The plunger face is removable so that various sizes and shapes can be used. A diameter of 36 mm. is convenient.

It is helpful to place a disk 38 mm. in diameter and 1.5 mm. thick on the bed of the apparatus, directly under the plunger so as to support the underside of the slice firmly in spite of warping or shrinkage.

*Determination.*—Mark the bed of the apparatus so that each slice may be placed in such a position that the plunger will act in the same relative place on each. Loosen the string. By means of the nut *B* and by pushing down on the counterweight raise the plunger. Insert



a slice of bread in the proper marked location. By means of the nut *B* adjust the pointers on both scales to zero in a uniform manner. Throw the electric switch, thereby starting the motor, which revolves the drum and depresses lever *G* at a uniform rate. When this lever is depressed to a predetermined point on scale *J* (that is, to a certain stress) the corresponding depression (or strain) is read on scale *D*. The stress chosen as the standard stopping point on scale *J* for any series of experiments is the largest which can be used without causing the pointer indicating strain on scale *D* to run off the scale. This point varies with the softness of the bread being tested. When the proper point has been reached the reading is taken on scale *D*. The further movement of the drum is arrested by grasping it with the fingers, and the switch is turned off. The drum is unwound to relieve the tension and to allow the levers to return to their normal positions.

This apparatus like the first is intended for the measurement of all slices in several loaves. The precision is approximately the same as that of the first apparatus. For this type of determination no stop watch is necessary.

This apparatus can be used for certain other types of measurement. For example concurrent readings of stress and strain may be made and curves drawn. Also the force on the plunger may be raised to a certain point, and this force maintained constant (as shown by the pointer on scale *J*) over a measured number of seconds by manual adjustment of the drum. Changes in the resulting depression or strain (as shown by the pointer on scale *D*) are plotted against time. Or the strain may be held constant and the resulting diminution in stress measured against time.

### **What Happens when Bread Crumb is Compressed**

The general characteristics of bread crumb under compression have been described in the references already given. At this time a little closer consideration of the mechanism is justified. We should be reminded that in testing the compressibility of bread crumb we are not testing one homogeneous material under one stress such as a mild steel bar under tension, for example. The structure of the bread evidently plays an important part. Considering this elaborate and delicate structure it is indeed surprising that bread recovers such a large percentage of its original form after ordinary compression. This shows that little permanent damage from tearing or breaking has been caused by the compression.

It is evident that in a compression test of a slice of bread only a part of the crumb subjected to stress is under compression. Some is subjected to bending stresses. Some parts of the slice, for example

the upper surface near the plunger, is actually under tension. The changes in the shapes of the cells under the action of the plunger are shown in Figures 7 and 8. In this case some slight deviation from normal conditions is caused by cutting away part of the slice in order to show the action directly under the center of the plunger.

In his book on "Elasticity, Plasticity and Structure of Matter" Houwink (1937) reproduces two diagrams from Burgers (1935). These show two strain-time diagrams resulting from the application and

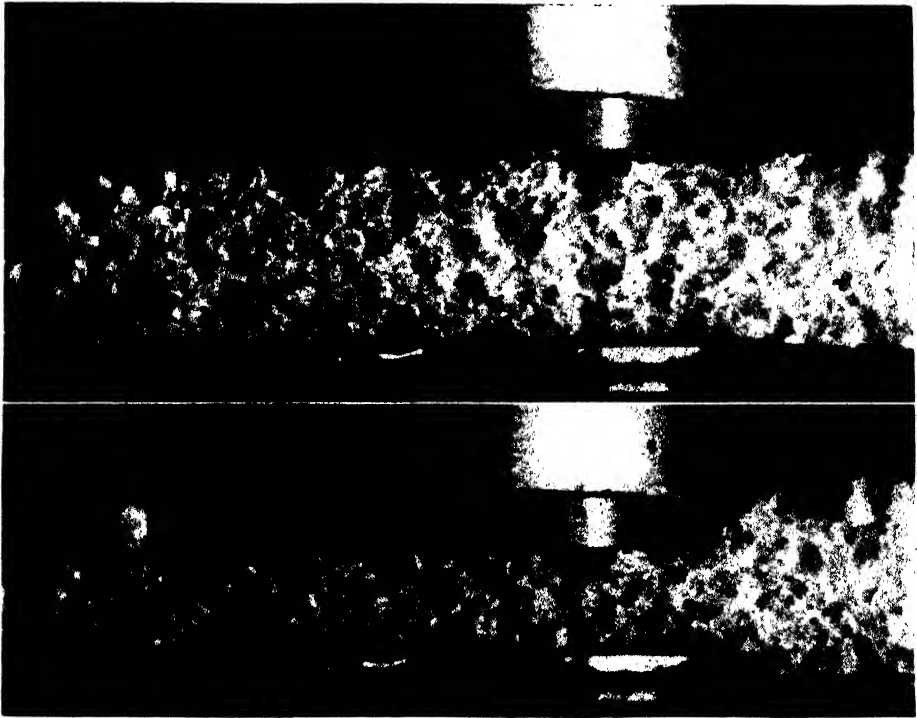


Fig. 7 (Above). Section of slice under plunger before compression.  
Fig. 8 (Below). Section of slice under plunger during compression. Same bread as shown in Figure 7.

subsequent removal of a stress to a system having both elastic and viscous properties. In the first instance the elastic and the plastic elements are connected in series. In the second instance they are connected in parallel. It is interesting and suggestive to note that the shape of the curve where the elastic and the viscous elements are connected in parallel closely approximates curves for bread crumb both for compression and for recovery, as for example in our Figure 2. It is evident that in the crumb both properties are present and that they can be measured separately as has been done by Halton and Scott Blair for dough. In the crumb the elastic properties greatly predominate.

By cutting a rectangular prism of bread of known size from a slice,

the compressibility of the separate piece can be determined without the influences of the other parts of the slice which are not under the plunger and the modulus of compressibility determined in absolute units. Results depend upon the conditions under which the test is made. Testing bread 48 hours old and allowing the force to act 1 minute, a modulus of  $6.5 \times 10^4$  was obtained. This compares with a modulus for dough obtained under different conditions and reported by Halton and Scott Blair (1937) of the order of  $4.0 \times 10^4$ . It must be remembered, however, that the dough was tested as an essentially continuous material, while the bread was tested with the open structure which is characteristic of bread crumb. A continuous mass of bread crumb material would of course be quite different in all physical characteristics.

### Effect of Several Variables on Compressibility

Compressibility measurements in the past have been primarily concerned with measurements of rate of staling. The effect of various factors other than staling will now be considered. All of the data here shown were derived from laboratory (but not pup) loaves baked in a pan of commercial size and shape. The standard formula and method are given below. This was varied as indicated for each factor studied. In all cases the amount of water was varied where necessary to give doughs of the same consistency.

FORMULA		
Sponge	Dough	Total, %
215 g. flour	135 g.	100
142 g. water	89 g. (variable)	66±
— powdered skim milk	21	6.0
— Sugar	17.5	5.0
— Salt	7.0	2.0
— Lard	14	4.0
5.25 g. yeast	—	1.5
1.75 g. yeast food	—	0.5

### Method

Sponge is mixed in batches taking 640 g. of flour at a time in a McDuffee-type dough mixer at low speed for 4 minutes. Temperature 85° F. Ferment 3 hours. Dough is mixed in McDuffee-type mixer at low speed 4 minutes. Stands 15 minutes, and is then mixed in Fleischmann mixer 3 minutes at 120 rpm. Dough is scaled at 18 oz., is put through the molder twice at 15-minute intervals, panned and proofed at 90° F. to constant height—usually 55 to 60 minutes, and baked in rotary oven for 35 minutes at 425° F.

The effect of varying the amount of skim-milk solids with appropriate variations in the added water is shown in Figure 9. Within the limits shown, increasing the skim-milk solids gives very significant increase in the softness of the crumb.

The effect of differences in the baking quality of the powdered skim-milk used is shown in Figure 10. This difference in the softness of the resulting bread still persists even when mixing methods were changed

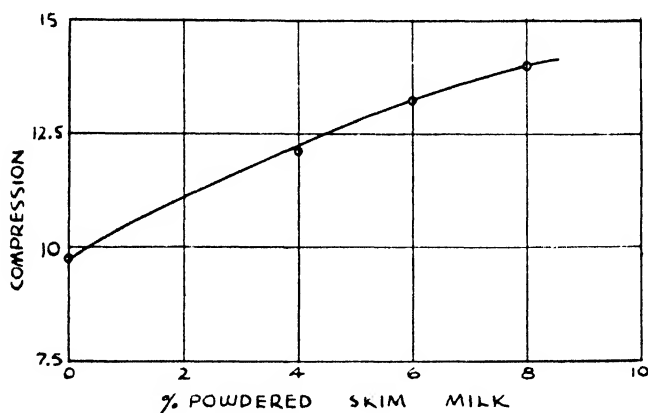


Fig. 9. Effect of increasing the percentage of powdered skim milk.

in a search for a method which would give better results with the poorer milk. Measurements of the softness of the bread could, in fact, be used as a method for measuring the comparative baking quality of two samples of the same bakery raw material.

In our opinion the fairest and most significant procedure for com-

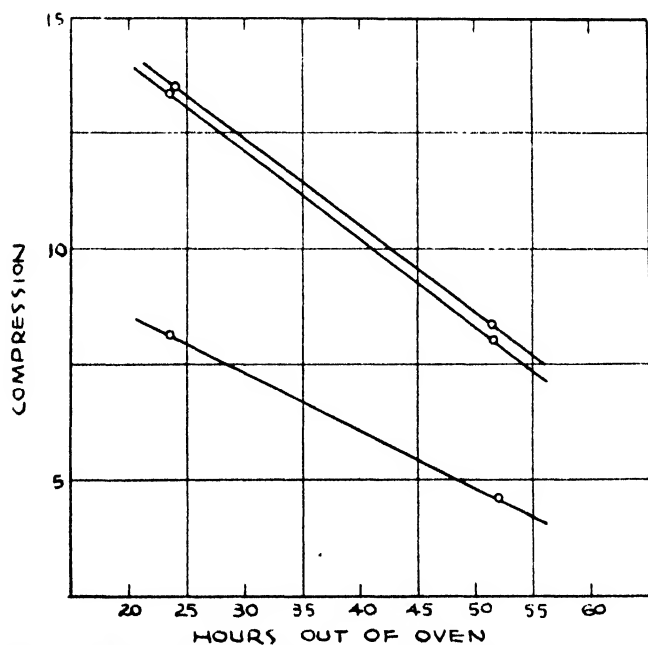


Fig. 10. Effect of powdered skim milk having good or poor baking quality. Each line represents bread made from a different lot of powdered milk. Each point is the average of all slices from three loaves.

paring baking quality of two doughs is to take conditions of absorption, mixing, and fermentation which are optimum for each, thus comparing each at its optimum. This is certainly the objective striven for in commercial baking.

TABLE III  
EFFECT OF VARIOUS FACTORS ON COMPRESSIBILITY OF BREAD

Fermentation		Time of bake	
Straight doughs	Compression	Time	Compression
Young (45 min.)	8.1	Short (25 min.)	16.3
Correct (2 hrs., 45 min.)	13.1	Medium (35 min.)	9.8
Old (4 hrs., 45 min.)	10.1	Long (45 min.)	8.6

Sugar		Shortening	
Amount	Compression	Amount	Compression
Low, 2%	13.0	Low (2% lard)	10.5
High, 6%	13.9	High (6% lard)	12.4

As is shown in Table III, increasing shortening causes a very significant increase in compressibility. An increase in sugar from 2% to 6% causes an increase in compressibility which is barely significant, if at all, when measured 20 hours out of the oven.

Compressibility is of course greatly affected by the degree of bake. Rather extreme conditions are shown in the same table. In this case the bread baked 25 minutes would have been considered barely salable. In the bread baked 45 minutes the crust was well browned, but not burned. When varying the amount of bake, it is *not* true of course that the softer the bread the better. Reference has already been made (Morison and Coriolis, 1929) to a method for measuring the degree of bake based upon the recovery of the crumb of bread after severe compression. The present apparatus could evidently also be used for setting up a standard as to when bread is sufficiently baked, and for measuring the adherence to such a standard.

To study properly the relation between fermentation and compressibility would require a comprehensive survey of different degrees of fermentation accompanied by various combinations of related conditions such as mixing, etc. No such extensive survey has yet been attempted. It seems probable, however, that a measurement of the compressibility of the crumb could be used as a guide to assist in approaching the optimum in fermentation, other conditions such as ingredients, degree of bake, etc., remaining constant. The effect of rather extreme conditions is shown in Table III. Maximum compressibility under conditions otherwise suitable seems to be associated with optimum fermentation.

The remarks just made regarding fermentation apply also to mixing. In the case of mixing, however, we have had somewhat more experience. Some of the results are shown in Figure 11. In this case compressibility

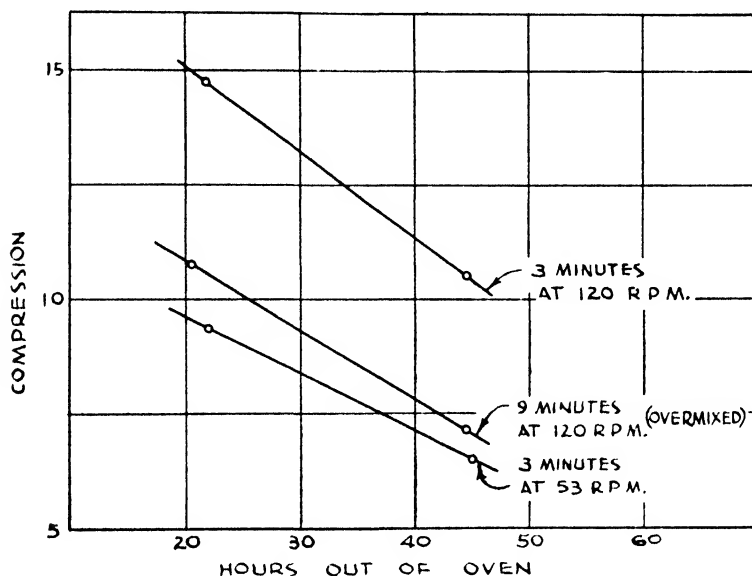


Fig. 11. Effect of mixing. Remix of sponge doughs given four minutes in a Hobart mixer at slow speed, plus added mixing in a Fleischmann mixer as shown. Each line represents bread from dough mixed as indicated. The superiority of the bread represented by the uppermost line is noticeable.

measurements first indicated that there was room for improvement in the texture of our laboratory loaves. This fact led directly to changes in mixing methods. Measurements of the compressibility of bread from dough mixed in different ways guided us to the optimum mixing now used. Further experiments in mixing gave no additional improvement in softness, affording a rational basis for the belief that the revised laboratory mixing method was near the optimum. It is suggested that measurements of compressibility while keeping all other factors constant, afford one of the best criteria for judging optimum mixing time.

### Conclusions

With proper refinements the compressibility test for bread crumb is capable of considerable precision. Some important refinements are described and a method for estimating the precision is given.

A new apparatus for carrying out this test is described, which gives simultaneous readings for stress and strain.

By the revised methods, compressibility tests can be carried out on commercially sliced bread. This test is therefore available as a commercial control. It can be used effectively to detect variations in texture from slice to slice and from loaf to loaf and to indicate the existence of lumps.

The following ingredients or procedures increase the softness of bread crumb, other factors being held constant:

Optimum mixing .....	High skim-milk solids. High baking quality of milk used.
Optimum fermentation .....	High sugar (to a very small extent).
Reduced amount baking .....	High shortening.

The baking quality of the skim-milk solids used has a marked effect on compressibility.

Other factors being held constant, compressibility can be used as measure of closeness of approach to optimum dough mixing. Unsuspected opportunities to improve dough-mixing conditions can often be detected by this test.

Rate of change in compressibility of bread has long been recognized as one of the best means for measuring rate of staling. By the improvements here suggested the significance of such measurements is greatly increased.

#### Literature Cited

- Bailey, C. H.  
1940 Physical tests of flour quality. Wheat studies of the Food Research Institute, Vol. 16, No. 6, Stanford Univ., Calif.
- Bailey, L. H.  
1930 A simple apparatus for measuring the compressibility of baked products. *Cereal Chem.* 7: 340-341.  
1932 The use of certain constituents in breadmaking with particular reference to the problem of staling. *Cereal Chem.* 9: 65-70.
- Baker, J. C.  
1939 Unpublished communication.
- Burgers, W. G.  
1935 First report on viscosity and plasticity. Academy of Sciences, Amsterdam, Holland.
- Cathcart, W. H., and Pushnik, W.  
1939 Effects of pan depths on twisted white pan bread. *Amer. Bakers' Assoc. Monthly Bul.* 15: 394-396.
- Davis, C. E.  
1921 Shortening, its definition and measurement. *Ind. Eng. Chem.* 13: 797.
- Halton, P., and Scott Blair, G. W.  
1937 A study of some physical properties of flour doughs in relation to their breadmaking qualities. *Cereal Chem.* 14: 201-219.
- Houwink, R.  
1937 Elasticity, plasticity and structure of matter. The Macmillan Co., Cambridge Univ. Press Dept., New York City, page 64.
- Katz, J. R.  
1917 Onderzoekingen naar het oudbakken worden van brood en de middelen van het te voorkomen. Published by Ministerium für Ackerbau, Industrie u. Handel, Holland.  
1928 Gelatinization and retrogradation of starch in relation to the problem of bread staling. Published as a chapter in R. P. Walton's book, "A Comprehensive Survey of Starch Chemistry." The Chemical Catalog Co., New York City.
- Morison, C. B., and Coriolis, E. G.  
1929 Application of heat to baking. The report of Amer. Gas Assoc. research at Amer. Inst. Baking. Original in Louis Livingston Library of Baking. Summary and conclusions published in *Bakers' Helper*, May 10, 1930.

- Platt, W.  
1930 Staling of bread. *Cereal Chem.* 7: 1-34.  
— and Fleming, R. S.  
1923 The action of shortening in the light of the newer theories of surface phenomena. *Ind. Eng. Chem.* 15: 390-394.  
— and Kratz, P. D.  
1933 Measuring and recording some characteristics of test sponge cakes. *Cereal Chem.* 10: 73-90.  
Scott Blair, G. W.  
1939 Psychorheology in the bread-making industry. *Cereal Chem.* 16: 701-711.  
Stateler, E. S.  
1936 Bread flavor and staling. *Proceedings Amer. Soc. Bakery Engineers*, pp. 139-141, Chicago, Illinois.  
Steller, W. R., and Bailey, C. H.  
1938 The relation of flour strength, soy flour and temperature of storage to the staling of bread. *Cereal Chem.* 15: 391-401.

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## MODIFICATION OF THE BAILEY-JOHNSON METHOD FOR MEASUREMENT OF GAS PRODUCTION IN FERMENTING DOUGH <sup>1</sup>

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(Read at the Annual Meeting, May 1940)

The measurement of gas production in fermenting dough has in recent years become a common practice in many cereal laboratories. Numerous and varied devices and procedures for such measurements have from time to time been described, such as those of Bailey and Johnson (1924), Kent-Jones (1939), James and Huber (1928), Fornet (1930), Jørgensen (1931), Markley and Bailey (1932), Blish, Sandstedt, and Astleford (1932), Elion (1933), Brabender (1934), Sandstedt and Blish (1934), Landis (1934), Eva, Geddes, and Frisell (1937), Malloch (1939) and Bailey (1939). Of these methods, the procedures of Sandstedt and Blish (1934) and Bailey and Johnson (1924) appear to have been most widely used.

Since 1932 apparatus for carrying out multiple determinations of gas production in fermenting doughs has been in use in several General Mills, Inc., laboratories. This equipment, similar in principle to that of Bailey and Johnson (1924), comprises a constant-temperature water bath and from 6 to 18 individual gasometers, depending upon the requirements of the particular laboratory. A detailed report of this apparatus, which has for many years been called a "Fermeter," has not previously been published, but it seems unnecessary to describe it completely in this paper since it is essentially similar to apparatus previously

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<sup>1</sup> Paper No. 17, Journal Series, General Mills, Inc., Research Laboratories.



reported by Bailey and Johnson (1924), Eva, Geddes, and Frisell (1937), and Bailey (1939).

The "Fermeter" and the apparatus described by Bailey (1939) differ only in certain minor particulars. We have preferred to use glass Mason jars as fermentation vessels, replacing the ordinary zinc top with one of spun brass of the same shape. A tubulature in the center of the lid is connected with heavy-wall rubber tubing leading to the gas-collecting burette. A cast lead washer (approximately 500 grams) is placed on the lid to keep the fermentation vessel immersed in the constant-temperature water bath.

The  $\text{CO}_2$  liberated during dough fermentation is collected in a 100-ml. burette with inverse graduation and a two-way stopcock. When the test is started the stopcock is set to collect the  $\text{CO}_2$  in the burette. By reversing the stopcock when the burette is filled, the gas can be discharged and the burette reset at zero, while keeping the fermentation vessel closed.

The above description will serve to show the fundamental principle of the Fermenter with reference only to certain details in which it differs from the apparatus described by Bailey.

The prime purpose of this paper, however, is not to describe the equipment but rather to describe a modification of the procedure for measuring gas production in fermenting doughs. Two years ago in this laboratory we modified the original Bailey and Johnson technique in several respects, developing a method of increased simplicity and convenience without loss in precision.

When the Fermeter was first used for measuring gas production, a normal 100-g. flour dough without sugar was mixed, fermented, and punched in the usual manner until three hours' fermentation time had elapsed. An aliquot of the dough representing 40 g. of flour was then placed in the Fermeter and gas production measured over a period of two hours. The value obtained with flours of satisfactory gassing power was of the order of 200 to 250 ml.

In carrying out the modified determination, 14 g. of flour is placed in the bottom of a half-pint Mason jar, preferably heaped on one side; 10 ml. of yeast suspension containing 4 g. of yeast per 100 ml. is pipetted into the jar and a smooth dough mixed with the aid of a small spatula. The lid is screwed firmly on the jar, which is placed in the constant-temperature water bath. The time of mixing is regarded as zero fermentation time.

The stopcock of the gas burette may be closed and gas collected from the beginning of fermentation or it may be left open during the initial two hours' fermentation to permit escape of carbon dioxide to the out-

side atmosphere. At the choice of the operator, determination of total gas production or production during any interval can be made.

It has been found that for most normal flours the rate of gas production during the first two hours of fermentation is essentially constant, and accordingly a satisfactory differentiation of gassing rate of various flours may be obtained by recording gas production during the last three hours of a five-hour fermentation period. A series of typical data is shown in Figure 1.

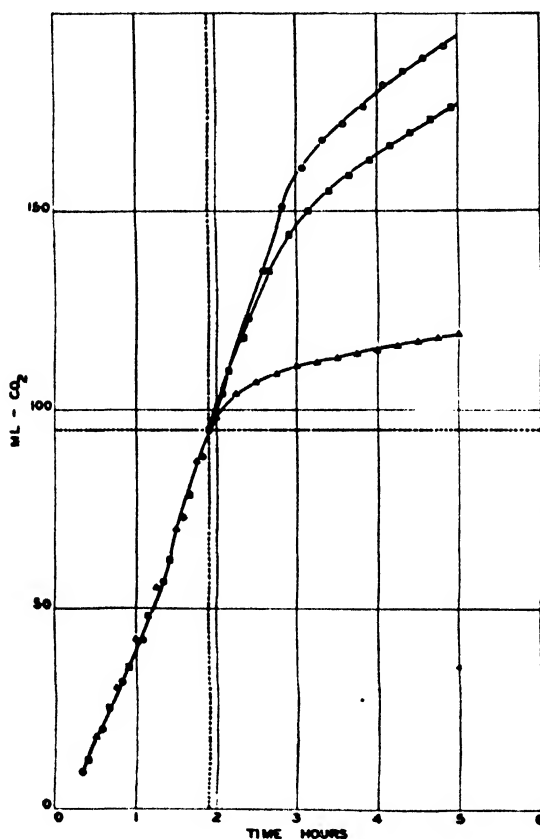


Fig. 1. Typical gas-production curves.

Since it has been found that small changes in pressure do not affect the rate of fermentation in doughs, the water level inside and outside the burette need be equalized only when readings are made, or when the capacity of the burette is reached and it must be discharged and reset.

For convenience in making the determination and recording values which can later be compared, a standard procedure has been adopted in our laboratories. The test is made with 14 g. of flour mixed into a

dough as described above. The dough is fermented in the constant-temperature bath at 30°C. After a lapse of two hours, during which gas is discharged to the atmosphere, the stopcock is closed and the gas produced is measured during the succeeding three hours. The gas production, measured in milliliters during this three-hour period, is recorded as the "Fermeter value" of the flour. Experience with a wide variety of flours, both natural and supplemented with malt flour, has shown that Fermeter values can be expected to range from 40 to 140 with a standard error of  $\pm 2.5$ .

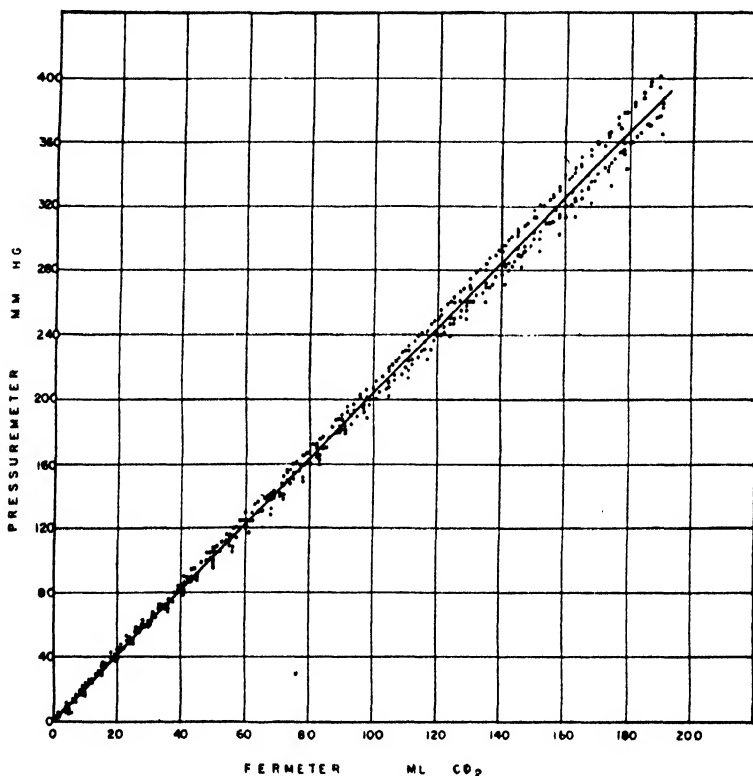


Fig. 2. Comparison of values secured with the Fermeter and with the pressuremeter.

It has been interesting to compare the "Fermeter value" with the pressuremeter values obtained by the Sandstedt and Blish procedure (1934). It has been shown by Eva, Geddes, and Frisell (1937) that measurements of gas production by the Bailey-Johnson and pressuremeter techniques are highly correlated. In order to ascertain the relationship between the gas-production values obtained in the pressuremeter and in the Fermeter, dough aliquots were scaled to provide the equivalent of 10 g. of flour for the pressuremeter and 14 g. of flour for the Fermeter.

Figure 2 shows the results of such comparative measurements. The individual points shown in this graph were obtained with 16 doughs, readings being taken on the Fermeter and on the pressuremeter at different time intervals. It will be seen that the correlation is extremely high. Calculation from the data above gives  $r = +.998$ . Results for the one method may be translated into terms of the other from the following regression equation calculated from these data: mm. mercury  $= 0.75 + 2.03$  (ml.  $\text{CO}_2$ ). Knowing the gas production value for a definite fermentation time interval for either the pressuremeter or Fermeter, one may calculate the corresponding value for the other method by use of the equation given above. The corresponding standard error of prediction, 6.9 mm. mercury, indicates that such translation of values may be done with satisfactory accuracy.

### Summary

A rapid volumetric method for determining rate of gas production in fermenting dough is described. When applied to flour, gassing power is reported as "Fermeter value," expressed in terms of  $\text{CO}_2$  evolved during the last three hours of a five-hour fermentation period.

Values obtained in the Fermeter are highly correlated with similar values obtained with the pressuremeter.

### Literature Cited

- Bailey, C. H.  
1939 Measuring fermentation rate and gas losses in dough. *Cereal Chem.* 16: 665-671.
- and Johnson, A. H.  
1924 Carbon dioxide diffusion ratio of wheat flour doughs as a measure of fermentation period. *Cereal Chem.* 1: 293-304.
- Blish, M. J., Sandstedt, R. M., and Astleford, G. R.  
1932 Sugars, diastatic activity, and "gassing power" in flour. *Cereal Chem.* 9: 378-393.
- Brabender, C. W.  
1934 Six years farinography. *Cereal Chem.* 11: 586-597.
- Elion, E.  
1933 A simple volumetric method for measuring gas production during dough fermentation. *Cereal Chem.* 10: 245-249.
- Eva, W. J., Geddes, W. F., and Frisell, B.  
1937 A comparative study of various methods of measuring flour gassing power. *Cereal Chem.* 14: 458-480.
- Fornet, Artur  
1930 Neue Apparate zur Prüfung von Mehl, Hefen, Backhilfsmitteln und Brot. *Technik u. Wirtschaft* 12: 260-261.
- James, T. R., and Huber, L. X.  
1928 Yeast fermentation in flour-water suspensions. *Cereal Chem.* 5: 181-191.
- Jørgensen, H.  
1931 On the separation of "gassing power" (diastatic activity) from "strength" in baking tests. *Cereal Chem.* 8: 361-374.
- Kent-Jones, D. W.  
1939 *Modern cereal chemistry* (3rd ed.), p. 585. Northern Publishing Co., Ltd., Liverpool.

- Landis, Q.  
1934 Diastatic activity in doughs and suspensions. *Cereal Chem.* 11: 24-35.
- Malloch, J. G.  
1939 A convenient apparatus for gas production determinations by the Blish method. *Cereal Chem.* 16: 178-182.
- Markley, M. C., and Bailey, C. H.  
1932 An automatic method for measuring gas production and expansion in doughs. *Cereal Chem.* 9: 591-594.
- Sandstedt, R. M., and Blish, M. J.  
1934 Yeast variability and its control in flour gassing power tests. *Cereal Chem.* 11: 368-383.

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## RELATIONS BETWEEN WHEAT MALT DOSAGE, FLOUR DIASTATIC ACTIVITY, AND GASSING POWER<sup>1</sup>

F. C. HILDEBRAND<sup>2</sup> and W. F. GEDDES<sup>3</sup>

(Read at the Annual Meeting, May 1940)

In recent years the use of malt supplements by the milling and baking industries has created a need for suitable methods of determining the activity of such materials. Leatherock, McGhee, and Giertz (1937) suggested that the relative activities of malted wheat flours could be estimated "by adding various percentages of the different malt flours to an untreated flour, and determining the maltose before and after treatment." Davis and Tremain (1938) elaborated upon this technique by comparing malt supplements in terms of the quantities required to give a definite level of response. These authors showed that such estimations of "malt value" could be made by adding varying increments of the material to be tested to a suitable untreated base flour, determining the diastatic activity or gas production, and estimating from graphs of the data thus secured the amount required to give an arbitrarily selected level of either measure. This technique was found to be necessary since a curvilinear relation exists between malt dosage and diastatic activity or gas production. Sandstedt (1938), employing the same malts at the dosages calculated by Davis and Tremain, obtained reasonably uniform gas-production values with a different base flour.

In their studies, Davis and Tremain found a high correlation (+.96) between "malt values" determined from diastatic activity and gas-production data but they noted that with some malts, and above a certain activity range with all treated flours, the evaluations based on diastatic activity were higher than would be predicted from the results of gas production measurements. However, for practical purposes, and

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<sup>1</sup> Paper No. 18, Journal Series, General Mills, Inc., Research Laboratories; No. 1816, Scientific Journal Series, Minnesota Agricultural Experiment Station.

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at the levels to which flours are generally treated, these discrepancies were considered to be of no great significance.

In the course of an investigation on the effects of wheat type, protein content, and malting conditions, respectively, on the properties of malted wheat flour (Geddes, Hildebrand, and Anderson, 1940), certain modifications were made in the technique proposed by Davis and Tremain which appear to the authors to afford greater simplicity and convenience. The data thus secured also provided the opportunity for a critical examination of the relative utility of diastatic-activity and gassing-power measurements as bases for the estimation of malt activity.

### Experimental

For the major studies, the experimental material comprised a series of 140 blends of 32 malted wheat flours with a Southwestern patent flour of low diastatic activity (166 mg. maltose per 10 g. flour). The malted wheat flours were experimentally produced during the course of an investigation by Geddes, Hildebrand, and Anderson (1940) and represented a wide range in amylase activity.

In addition, a second and a third series were prepared. In the second series, the lower-activity malts from the group referred to above were composited and varying amounts of the composite added to the base flour previously mentioned. In the third series, varying increments of a commercial malted wheat flour were added to a different Southwestern patent flour, also of low diastatic activity (130 units).

Gas-production determinations were carried out in duplicate in an apparatus similar to that described by Sherwood, Hildebrand, and McClellan (1940) using 14 g. flour and 10 ml. yeast suspension containing 0.42 g. fresh compressed yeast. The total gas production for zero to five hours was recorded. Diastatic activity was determined in duplicate by the ferricyanide procedure as modified by Sandstedt (1937). All weighings of malts and blends were made on a 13.5% moisture basis.

In the first series, each of the malted wheat flours was added to the common base flour at levels of 0.25%, 0.50%, 0.75%, and 1.00% and diastatic-activity and gassing-power determinations were carried out on the resulting blends. In the instance of 12 of the malts which were found to be of low activity, additional determinations were made on blends containing 2.5% malt flour. In the second series, the composite of the low-activity experimental malts was added to the base flour previously mentioned in 0.2% increments from 0.2% to 3.2%. In the third series, the commercial malted wheat flour was added to a different Southwestern base flour at several dosage levels varying between 0.025% and 1.75%.

### Results

The relation between malt-flour dosage and gas production was found to be consistently nonlinear, an observation which is in accord with that of Davis and Tremain (1938). Examination of the data, however, revealed that gas production was proportional to the logarithm of the dosage. Typical examples of these relationships are shown in Figure 1, which also includes the data for a larger number of dosages described above as the second and third series.

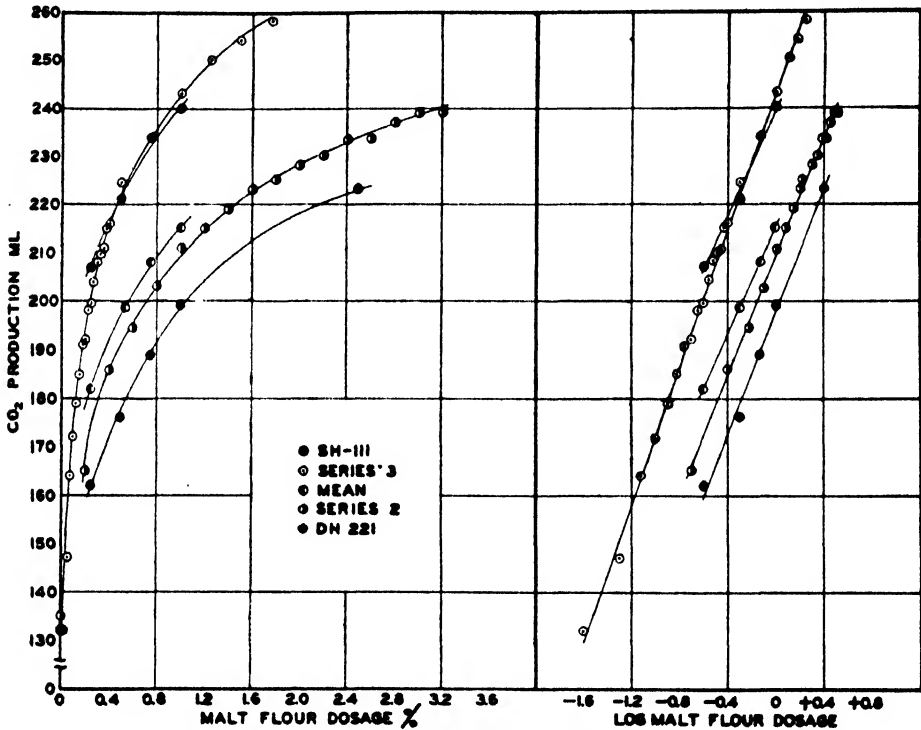


Fig. 1. Relation between malt flour dosage and gas production.

Diastatic activity also was found to bear a curvilinear relationship to dosage, as shown in Figure 2. In this instance, however, the relation was not a logarithmic one but was best expressed by a quadratic equation of the general form  $D = a + bx - cx^2$  where  $D$  is the diastatic activity and  $x$  the dosage.

In order to provide as accurate a basis as possible for estimating the dosage of each malt required to produce a given level of diastatic activity or gassing power, regression equations were calculated for the duplicate sets of determinations for each malt. In computing these equations from the gassing-power data, the labor involved was materially reduced by expressing the dosages as logarithmic functions and calculating the

appropriate linear regressions. Unfortunately, this procedure, as has already been shown, could not be used with the diastatic-activity data and accordingly it was necessary to fit individual quadratic equations.

As a basis for estimating malt activity, a level of 280 mg. of maltose was selected for calculations based on the diastatic-activity data. In

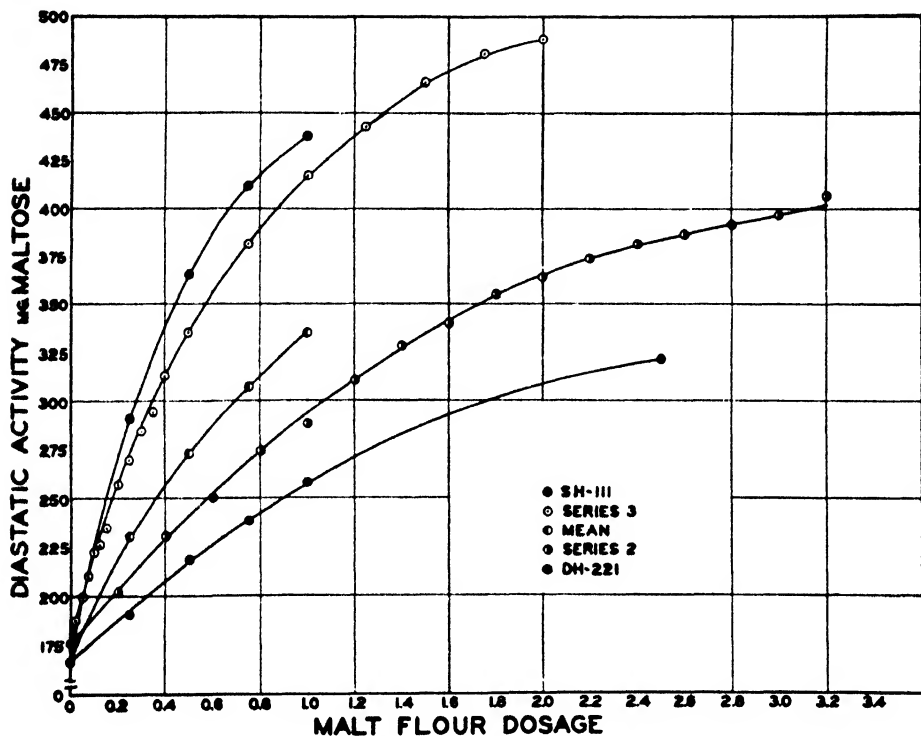


Fig. 2. Relation between malt flour dosage and diastatic activity.

order to establish an equivalent level of gassing power, the regression equation for gassing power on diastatic activity—again a curvilinear relationship—was calculated for the major series of 140 blends. From the equation thus computed, it was found that with the particular base flour employed and under the conditions of our determinations, a level of 204.7 ml.  $\text{CO}_2$  was equivalent to 280 mg. maltose.

The amount of each malt required to give the above levels of diastatic activity and gas production was computed from the respective individual regression equations for these two measures on malt-flour dosage. In this manner, duplicate estimates of the malt dosages required to give the selected levels of both diastatic activity and gas production were secured. The means of these duplicate determinations are recorded in Table I. Casual inspection of these values indicates fairly satisfactory agreement between the dosages estimated from the two types of meas-



TABLE I

MALT FLOUR DOSAGE TO GIVE 280 MG. MALTOSE OR 204.7 ML. CO<sub>2</sub>—  
MEANS OF DUPLICATE DETERMINATIONS

Treat- ment <sup>1</sup>	Hard red spring				Amber durum			
	High protein		Low protein		High protein		Low protein	
	DA <sup>2</sup>	GP <sup>2</sup>	DA	GP	DA	GP	DA	GP
	%	%	%	%	%	%	%	%
111	0.21	0.23	0.28	0.30	0.27	0.32	0.24	0.30
112	0.20	0.25	0.24	0.30	0.28	0.31	0.27	0.29
211	0.29	0.35	0.30	0.31	0.49	0.46	0.45	0.45
212	0.30	0.30	0.29	0.28	0.44	0.49	0.40	0.40
121	0.86	0.88	1.53	1.53	0.74	0.77	0.86	0.93
122	1.27	1.17	1.52	1.54	0.89	0.87	1.10	1.04
221	1.29	1.40	1.37	1.35	1.30	1.30	1.64	1.39
222	1.56	1.55	1.51	1.59	1.41	1.31	1.29	1.38

<sup>1</sup> Malting treatment—cf. Geddes, Hildebrand, and Anderson (1940).

<sup>2</sup> DA = dosage calculated from diastatic activity data; GP = dosage calculated from gas production data.

urement. The data were analyzed statistically and certain of the pertinent constants are given in Table II. The high correlation (+.992) between the paired values for each malt shows that the two types of measurement give closely parallel results; moreover the difference between the mean values for the two methods over all malts of 0.006% is not statistically significant. The relative precision of the two methods

TABLE II

COMPARISON OF DIASTATIC ACTIVITY AND GAS PRODUCTION MEASUREMENTS  
AS BASES FOR MALT FLOUR EVALUATION

	Dosage calculated from	
	DA data	GP data
	%	%
Mean	0.786	0.792
Mean difference between duplicates	0.0421	0.0429
Standard error of single determination	0.0527	0.0469
Correlation between dosages by each method: $r = +.992$ (1% pt = 0.449)		

for estimating malt dosage is given by a comparison of the respective standard errors; these were tested and found not significantly different. Taking the average standard error as approximately 0.05%, the precision of estimate for both methods is roughly 0.1%. In other words if the means of duplicate determinations for two malts do not differ by this amount, they would be regarded as being of equal activity. This accuracy can hardly be regarded as satisfactory in the instance of active

malts which need be added only in small amounts. However, the precision with which malt dosage can be estimated increases with increasing malt activity. Thus for the active malts, 111 to 212 inclusive, the standard error for both methods of estimation is 0.017% as compared with 0.068% for the less active malts 121 to 222 inclusive. Thus for malts requiring dosages of from 0.20% to 0.45% to produce a flour of 280 units of diastatic activity, the precision is increased to within 0.034%.

In order to test the reliability of this general technique for estimating malt dosage, the percentages of each malted wheat flour computed from the gas-production data shown in Table I were added to the base flour previously employed. Gas-production determinations were carried out in duplicate on the resulting blends, and the mean values for each blend are recorded in Table III. The mean gas production over all blends is

TABLE III  
MEAN GAS-PRODUCTION VALUES FOR FLOUR BLENDS DIASTATED WITH  
EQUIVALENT QUANTITIES OF MALTED WHEAT FLOURS

Treatment	Hard red spring		Amber durum	
	High protein	Low protein	High protein	Low protein
	<i>ml.</i>	<i>ml.</i>	<i>ml.</i>	<i>ml.</i>
111	205	207	207	208
112	207	206	208	205
211	205	206	207	204
212	205	203	205	204
121	207	206	205	206
122	206	205	203	205
221	206	206	204	206
222	206	207	203	206
Mean = 205.5 ml.				

205.5 ml., which agrees closely with the calculated level of 204.7 ml. of CO<sub>2</sub>. Moreover, the variation between blends was less than that between duplicates (standard error of a single determination 1.8 ml.). It may therefore be concluded that the technique employed provides a highly satisfactory means of estimating malt dosage and also therefore of measuring relative malt activities.

While it has been shown that either gas-production or diastatic-activity data may be used interchangeably in estimating malt dosage under the conditions described above, such alternate use is contingent upon the selection of equivalent levels of these measures. In this connection it is of interest to examine the relationship between diastatic activity and gas production found in this study. The paired values for the 140 blends of the first series are shown in Figure 3.

While the correlation based on the assumption of a linear relationship is quite high ( $r = +.954$ ; 1% pt. = 0.213 approximately) it is clearly evident that the regression is curvilinear. After several trials in fitting various types of curves, it was found that the data were best represented

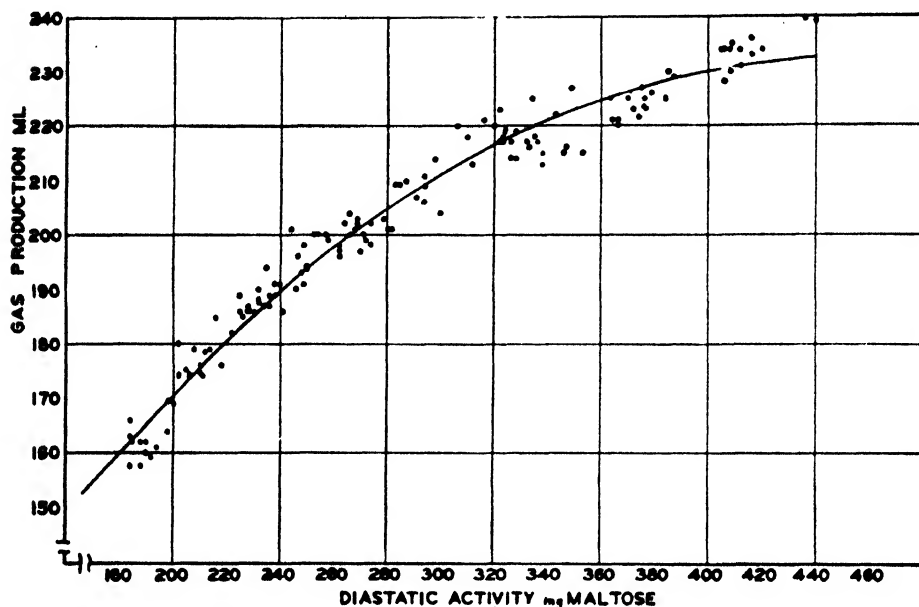


Fig. 3. Relation between diastatic activity and gas production for flour blends made by adding increments of malted wheat flours to a common base flour.

by a quadratic equation of the general form  $G = a + bD - cD^2$  where  $G$  is gas production,  $D$  is diastatic activity, and  $a$ ,  $b$ , and  $c$  are constants. The curve thus fitted is that given in Figure 3. When allowance was made in this manner for nonlinearity the correlation coefficient between these variables was increased to  $+ .979$ .

### Discussion

The data presented show that if suitable care is taken to select equivalent levels of diastatic activity and gas production, malt dosage may be estimated from either measure interchangeably and with equal precision, although the use of diastatic-activity data is much less convenient. In the case of gas-production values, linear regressions may be calculated after expressing malt dosage as a logarithmic function, whereas with diastatic-activity measurements, calculations of quadratic equations are necessary—a procedure which is much more laborious.

In the routine application of this technique, it is altogether unlikely that one would wish to resort to the calculation of individual regression equations. Indeed, where gas-production measurements have been employed it would appear that one could dispense with such calculations

without material loss in accuracy. If dosage levels are expressed as logarithms, and are plotted against the corresponding gas productions, a straight line may be drawn through the points with reasonable accuracy; if necessary the required dosage may be determined by extrapolation.

In working from a curvilinear relation, a number of determinations must be carried out in order to establish accurately the form of the curve, or a smaller number must be made at levels of activity falling close to and on both sides of the arbitrarily selected standard activity level. Since such procedures necessarily involve a relatively large number of analyses, the use of logarithmic plotting suggested above seems to promise increased convenience and simplicity to attain equivalent accuracy.

In recent years several papers have appeared in the literature dealing with the relation between diastatic activity and flour gassing power and it is becoming increasingly evident that an exact parallelism does not exist between these variables—formerly regarded by many workers as synonymous for practical purposes. Thus it has been shown that the relation between these measures is influenced by variations in substrate, such as for example in sucrose content (Blish, Sandstedt, and Astleford, 1932), and in starch susceptibility (Karacsonyi and Bailey, 1930). Other workers (Landis and Frey, 1936; Bottomley, 1938; Fisher, Halton and Hines, 1938), have suggested that the different conditions under which the two tests are carried out is an important factor affecting the correlation between these measures. In the light of these views, the direct comparison of gas production and diastatic values shown in Figure 3 is of particular interest since the measurements were made upon essentially the same substrate. Actual determinations of sucrose and reducing sugars made on a number of the flour blends selected to represent extremes of malt activity and dosage, showed that there was no measurable variation in sugar content. Accordingly it must be concluded that factors in addition to variations in substrate operate to cause a variable relation between diastatic activity and gassing power. Even with an extremely uniform substrate, the increase in gas production per unit increase in diastatic activity becomes progressively less with increasing activity. This lends further emphasis to the views advanced by several workers, as for example Landis and Frey (1936) and Eva, Geddes and Frisell (1937), that these two measures cannot be regarded as synonymous.

### Summary

Thirty-two experimentally produced malted wheat flours of widely varying amylase activity were evaluated by a technique similar to that suggested by Davis and Tremain (1938). The malt flours were blended

at varying levels with a common base flour of low diastatic activity, and diastatic-activity and gassing-power determinations were carried out on the resulting blends.

Gas production for any given malt was found to vary directly as the logarithm of the dosage, while the relationship between dosage and diastatic activity was best expressed by a quadratic equation.

The amount of each malt required to produce 280 mg. maltose and the equivalent level of five-hour gas production (204.7 ml.  $\text{CO}_2$ ) was calculated from the individual regression equations. The dosages thus calculated from diastatic-activity and gassing-power measurements were not significantly different and the precisions of the two techniques were equal. The precision of estimate increases with increasing malt activity; for malts requiring dosages of from 0.20% to 0.45% the precision is within 0.34%.

Flour blends prepared with dosages estimated from gassing-power data gave a mean production of 205.5 ml. of  $\text{CO}_2$ , the difference in the values for the individual malt flours being less than the error in the determination of gas production.

Estimation of malt activity is most conveniently and simply made from gas-production data by plotting ml.  $\text{CO}_2$  against the logarithm of dosage. The quantities of malt required to produce the selected level of gassing power may be read from the straight line thus obtained.

The relation between diastatic activity and gassing power was curvilinear, although the substrate was essentially the same throughout. This observation supports the view that the different conditions under which the two tests are carried out is an important factor, aside from variations in sucrose content and starch susceptibility, affecting the relation between these measures.

#### Acknowledgments

The authors are indebted to the personnel of the Works Projects Administration, Official Project No. 65-1-71-140, sponsored by the University of Minnesota and the personnel of the National Youth Administration for assistance in carrying out the experimental work.

#### Literature Cited

- Blish, M. J., Sandstedt, R. M., and Astleford, G. R.  
1932 Sugars, diastatic activity and "gassing power" in flour. *Cereal Chem.* 9: 378-393.
- Bottomley, R. A.  
1938 The relationship between diastatic activity (maltose figure) and "gassing power" of experimentally milled flours from some Australian wheats. *Cereal Chem.* 15: 509-520.
- Davis, C. F., and Tremain, H. E.  
1938 A comparison of methods for evaluating the diastatic properties of malt as a flour supplement. *Cereal Chem.* 15: 826-831.

- Eva, W. J., Geddes, W. F., and Frisell, B.  
1937 A comparative study of various methods of measuring flour gassing power. *Cereal Chem.* **14**: 458-480.
- Fisher, E. A., Halton, P., and Hines, S. F.  
1938 Some observations on maltose production in flour and dough. *Cereal Chem.* **15**: 363-377.
- Geddes, W. F., Hildebrand, F. C., and Anderson, J. A.  
1940 The effect of wheat type, protein content and malting conditions on the properties of malted wheat flour. *Cereal Chem.* **17**: —.
- Karacsonyi, L. P., and Bailey, C. H.  
1930 Relation of the overgrinding of flour to dough fermentation. *Cereal Chem.* **7**: 571-587.
- Landis, Q., and Frey, C. N.  
1936 The enzymes of flour in relation to yeast fermentation. *Cereal Chem.* **13**: 281-317.
- Leatherock, L. E., McGhee, H. W., and Giertz, J. W.  
1937 Diastatic activity in connection with flour mill control. *Cereal Chem.* **14**: 161-181.
- Sandstedt, R. M.  
1937 The adaptation of the ferricyanide maltose method to high diastatic flours. *Cereal Chem.* **14**: 603-604.  
1938 The relation of alpha- and beta-amylases to the evaluation of malts in flour technology. *Cereal Chem.* **15**: 832-835.
- Sherwood, R. C., Hildebrand, F. C., and McClellan, B. A.  
1940 Modification of the Bailey-Johnson method for measurement of gas production in fermenting dough. *Cereal Chem.* **17**: 621-626.

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## COMPARISON OF METHODS FOR THE DETERMINATION OF PROTEOLYTIC ACTIVITY. II. STUDIES ON MALTED WHEAT FLOURS<sup>1</sup>

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(Read at the Annual Meeting, May 1940)

Early studies of methods of estimating proteolytic activity in cereal products have been briefly reviewed by Hildebrand (1939). In an investigation reported at that time, the author compared rate-of-gelation, viscometric, and formol titration procedures. It was concluded that the two methods first mentioned gave results appreciably different from techniques based on the measurement of proteolysis end products by formol titration, presumably because dipeptidase and/or polypeptidase activity had an appreciable effect on the results obtained by the formol titration methods, whereas with the rate-of-gelation and viscometric procedures proteinase activity alone was estimated. As between the two latter methods, the rate-of-gelation technique seemed preferable since it gave better differentiation between samples, although it is not well adapted to general use because of its requirement of relatively elaborate and costly apparatus.

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<sup>1</sup> Paper No. 16, Journal Series, General Mills, Inc., Research Laboratories. Subcommittee report, 1939-40, Committee on Methods of Analysis.

Recently Ayre and Anderson (1939) have modified a technique suggested by Northrup (1932) and have applied the method in its modified form to the determination of proteolytic activity in barley malts. This method is based on autolytic digestion of the material to be tested, precipitation of the protein with trichloroacetic acid after one and three hours' digestion and determination of the unprecipitated nonprotein nitrogen by a Kjeldahl procedure. The increase in nonprotein nitrogen is taken as a measure of proteolytic activity.

The experiment here reported was planned to afford a comparison between the Ayre and Anderson procedure and the rate-of-gelation method of Landis and Fry (1938), using malted wheat flours as the material investigated.

### Experimental

Twelve samples of experimentally produced malted wheat flours, selected so as to give a reasonably wide range of proteolytic activity, were used in this study. These samples were portions of the material investigated by Geddes, Hildebrand, and Anderson (1940). A complete description of the origin of these samples is given in the paper cited.

TABLE I  
PROTEOLYTIC ACTIVITY OF MALTED WHEAT FLOURS

Sample	Landis and Frey method—milliunits per g.	Ayre and Anderson method—mg. nonprotein N per 100 g.
ML 211	0.81	64
	0.69	68
DH 222	1.09	95
	1.03	102
ML 121	1.07	70
	1.31	74
MH 212	1.29	111
	1.40	106
DL 211	1.66	143
	1.65	133
MH 122	1.71	111
	1.63	119
DH 211	1.65	131
	1.75	129
ML 112	1.80	169
	1.98	167
DH 122	1.97	155
	1.96	147
DL 112	2.07	201
	2.14	211
DL 111	2.16	191
	2.20	163
DH 111	2.98	251
	2.95	241

The Ayre and Anderson procedure as outlined in their original presentation was used without modification. The Landis and Frey (1938) rate-of-gelation technique was employed embodying the modifications suggested by Hildebrand (1939).

The results obtained are shown in Table I. In order to facilitate comparison, the results by both methods were reduced to the same numerical basis. The ratio between the mean values for all samples was computed and the data for the Ayre and Anderson method were multiplied by the factor thus obtained. The data recalculated in this fashion

TABLE II  
PROTEOLYTIC ACTIVITY OF MALTED WHEAT FLOURS  
(Values reduced to same numerical basis)

Sample	Landis and Frey method—milliunits per g.	Ayre and Anderson method—mg. nonprotein N per 1.22 g.
ML 211	0.81 0.69	0.78 0.83
DH 222	1.09 1.03	1.16 1.25
ML 121	1.07 1.31	0.86 0.90
MH 212	1.29 1.40	1.36 1.29
DL 211	1.66 1.65	1.75 1.62
MH 122	1.71 1.63	1.36 1.45
DH 211	1.65 1.75	1.60 1.58
ML 112	1.80 1.98	2.06 2.04
DH 122	1.97 1.96	1.89 1.80
DL 112	2.07 2.14	2.46 2.58
DL 111	2.16 2.20	2.33 1.99
DH 111	2.98 2.95	3.07 2.94

are given in Table II. Table III shows estimations of the error and differentiation between samples given by the two methods.

### Discussion

While it is evident that the number of samples tested is insufficient to permit drawing of more than tentative conclusions, nevertheless the data serve for a preliminary estimation of the correlation between the two procedures and should be of value in guiding future studies.



TABLE III

	Landis and Frey method	Ayre and Anderson method
Mean, all samples	1.706	1.706
Range	0.69-2.98	0.78-3.07
Variation between samples, mean square	7.6033	9.3770
Variation between duplicates, mean square	0.0720	0.0989
<i>F</i>	115.1	103.4
Correlation between results by both methods	$r = +.956$	

The high degree of correlation between results indicates that both procedures measure the same type of activity. It has been assumed (Landis and Frey, 1938; Hildebrand, 1940) that the rate-of-gelation technique affords an estimate of proteinase activity which is substantially unaffected by the presence of other proteolytic enzymes. On the basis of the results given in this study, the same assumption may be made for the Ayre and Anderson procedure.

It may be seen from the values given in Table III that the experimental error of the two procedures is essentially the same. The difference between duplicate errors is too small to be significant with the limited amount of data employed. Similarly, it may be seen from the *F* values that both methods have approximately the same power to differentiate between samples. Since the performance of both methods is essentially the same, the Ayre and Anderson technique seems preferable because of its comparative simplicity and convenience.

### Summary

The Landis and Frey rate-of-gelation and Ayre and Anderson precipitation methods for proteolytic activity have been compared, using twelve experimentally produced samples of malted wheat flour.

Both methods give essentially similar results and have approximately the same experimental error and ability to differentiate between samples.

The Ayre and Anderson technique is to be preferred because of its greater simplicity and convenience.

### Acknowledgment

The author is indebted to Philip M. Sautier for assistance in the studies with the Ayre and Anderson method.

### Literature Cited

Ayre, C. A., and Anderson, J. A.

1939 Varietal differences in barleys and malts. VI. Autolytic proteolytic activity of malt and its correlations with wort nitrogen and barley nitrogen fractions. *Can. J. Research* **17C**: 239-246.

- Geddes, W. F., Hildebrand, F. C., and Anderson, J. A.  
1940 The effect of wheat type, protein content, and malting conditions on the properties of malted wheat flour. *Cereal Chem.* 17: (in press).
- Hildebrand, F. C.  
1939 A comparison of methods for the determination of proteolytic activity. *Cereal Chem.* 16: 792-798.
- Landis, Q., and Frey, C. N.  
1938 A sensitive method for the determination of proteolytic activity. *Cereal Chem.* 15: 91-101.
- Northrup, J. H.  
1932 Crystalline pepsin. IV. Hydrolysis and inactivation by heat. *J. Gen. Physiol.* 16: 33-40.

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## COLLABORATIVE STUDY OF A RAPID METHOD FOR THE DETERMINATION OF FLOUR PIGMENTS<sup>1</sup>

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(Read at the Annual Meeting, May 1940)

The determination of carotenoid pigments in flour has generally been conducted by extraction with a mixture of naphtha and absolute ethyl alcohol (93 parts to 7 parts) according to the methods of Ferrari (1933), or Geddes *et al.* (1934), as outlined in *Cereal Laboratory Methods* (1935). According to these procedures, overnight standing (16 to 18 hours) is required to obtain complete extraction, and the method is thus not suited to purposes of mill control. A rapid procedure involving high speed stirring has been described by Coleman and Christie (1926) but does not appear to have met with general favor. Recently, Binnington, Sibbitt, and Geddes (1938) suggested the use of water-saturated n-butyl alcohol as a solvent, and Binnington and Geddes (1939) describe a rapid method for flour, involving the use of this solvent in conjunction with a 15-minute extraction period. This method yields results of a higher order than the naphtha + alcohol procedure, thus necessitating a mental adjustment on the part of the operator to a higher scale of values. The rapidity and convenience of the procedure, however, are such as to suggest its wider employment, and the Committee on Methods of Analysis therefore undertook a collaborative study of this method.

Nine collaborators participated, and three samples of bleached and three of unbleached flour were circulated. Detailed directions for preparation of the extracts were supplied; in the instance of the new method these followed the authors' procedure and in the case of the naphtha + alcohol technique, which was employed as a control, the

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<sup>1</sup> Subcommittee report, 1939-40 Committee on Methods of Analysis. Paper No. 21, *Journal Series, General Mills, Inc., Research Laboratories.*

official A. A. C. C. procedure was followed. Specific directions for preparation of the extracts *only* were detailed, and each collaborator was requested to employ whatever method was available for estimating the pigment content of the extracts so obtained. Whenever possible, it was requested that more than one method be employed, and it was further suggested that a visual comparison against potassium chromate standards, using mercury vapor illumination, be used if facilities permitted. In addition, it was requested that duplicate or triplicate determinations be carried out on each sample.

The pigment content of flour, whether bleached or unbleached, is subject to reduction upon storage, and as all collaborators could not be expected to analyze the samples at the same time, steps were taken to secure an estimate of such losses in the instance of the flours employed in this study. For this purpose, the samples were stored at room temperature in cans with tight-fitting lids, tests being conducted from time to time. With water-saturated butanol, the drop in pigment content did not exceed 0.10 ppm. at the end of 30 days' storage, and with naphtha + alcohol, a maximum of 0.12 ppm. was found in 44 days. With the exception of one collaborator, all tests were conducted within 28 days and these results may therefore be considered to be substantially free from error due to alteration of the sample. One collaborator was unable to analyze the samples until a 61-day period had elapsed, and since his results suggested that some loss of pigment had occurred, they have been omitted from the statistical comparisons. Several other groups of results were also eliminated upon the collaborators' own statement that the accuracy was doubtful due to difficulties in calibrating and standardizing the instruments employed. For these reasons, the summarized results and statistical analyses presented are based upon the means for two collaborators in the instance of each method of estimation.

Because of the fact that various methods of estimating the pigment content of the extracts were employed by the different collaborators, it is possible to examine the results not only from the standpoint of solvents and extraction procedures, but also with reference to methods of estimation as well.

In Table I, the mean results for each flour are presented, together with certain statistical comparisons.

The spectrophotometric values for which means are shown in Table I were secured with a König-Martens spectrophotometer equipped with a mercury-vapor light source and a Bausch and Lomb Martens-type photometer, mercury-vapor light source, and Corning filter isolating the 4358 A.U. line of the mercury spectrum. Both sets of readings

TABLE I  
COMPARISON OF SOLVENTS AND OF METHODS OF ESTIMATING  
PIGMENT CONTENT OF EXTRACTS—MEAN VALUES

Flour	Water-saturated butanol				Naphtha + alcohol (93 : 7)			
	Spectro- photo- meter	Visual	Photo- electric	Mean	Spectro- photo- meter	Visual	Photo- electric	Mean
A	0.84	0.77	0.86	0.82	0.62	0.55	0.52	0.56
B	1.61	1.45	1.61	1.56	1.14	1.04	1.17	1.09
C	1.10	0.95	1.00	1.02	0.74	0.65	0.70	0.68
D	2.94	2.78	3.03	2.92	2.31	2.11	2.36	2.23
E	3.38	3.30	3.53	3.40	2.71	2.52	2.76	2.62
F	3.45	3.27	3.62	3.45	2.77	2.58	2.86	2.69
Mean	2.22	2.09	2.28	—	1.72	1.58	1.73	—

Correlation between results by water-saturated butanol and naphtha + alcohol extractions:  $r = +.9925$  (1% pt. = 0.212 approx.)

Regression equations:

Carotene ppm. (butanol) =  $0.21 + 1.219$  carotene ppm. (naphtha + alcohol).

Carotene ppm. (naphtha + alcohol) =  $0.808$  carotene ppm. (butanol)  $-0.15$ .

Standard error of prediction:

Carotene (butanol) from carotene (naphtha + alcohol) =  $\pm 0.14$  ppm.

Carotene (naphtha + alcohol) from carotene (butanol) =  $\pm 0.11$  ppm.

were made at 4358 A.U., the transmittancies obtained being computed to carotene employing the values 1.6632 and 1.91565 for the specific transmissive indices of carotene in water-saturated butanol and naphtha + alcohol respectively.

Visual results were obtained by matching against potassium chromate standards, employing unfiltered mercury arc or vapor radiation as an illuminant, and using a standard series procedure for matching according to the technique outlined in Cereal Laboratory Methods (1935).

Both sets of photoelectric results were secured with Evelyn photoelectric colorimeters equipped with glass filters having a peak transmission at 440 millimicrons and a transmission range of approximately 60 millimicrons. These instruments were standardized against a spectrophotometer using flour extracts, the same calibration curve being employed for both.

In addition to the results summarized in Table I, two collaborators reported values secured with KWSZ photoelectric photometers. These results differ widely from the mean values tabulated above and will be considered separately.

The results obtained in this study may be conveniently considered under two headings: (1) comparison between solvents, and (2) comparison between methods of estimation.

Comparing solvents, it will be noted that the rapid method yields results of a definitely higher order of magnitude and thus direct comparisons are not possible. The extremely high correlation between the two procedures indicates that both techniques give relatively similar results. In order to ascertain whether the relation between values given by the two solvents is affected by variation in method of estimating pigment content or by differences between laboratories, regression coefficients were calculated for individual sets of data. In no case was there a significant difference.

It is of further interest to compare the general equation relating values obtained by the two solvents studied with that given by Binnington, Sibbitt, and Geddes (1938). In the present study the equation derived is:

Carotene ppm. (butanol) =  $0.21 + 1.219$  carotene ppm. (naphtha + alcohol)  
while the latter authors, working with a series of 150 hard red spring wheat flours obtained the expression:

Carotene ppm. (butanol) =  $0.14 + 1.2377$  carotene ppm. (naphtha + alcohol)

Table II shows a series of data selected at random, which affords a comparison between these two equations.

TABLE II  
COMPARISON OF OBSERVED VALUES WITH VALUES COMPUTED  
FROM TWO REGRESSION EQUATIONS<sup>1</sup>

Naphtha + Alcohol	Butanol		Observed
	Equation No. 1	Equation No. 2	
	<i>Carotene p.p.m.</i>		
0.50	0.82	0.76	0.85
0.60	0.95	0.88	0.78
0.63	0.98	0.92	0.91
0.70	1.06	1.01	1.05
0.74	1.11	1.06	1.06
0.74	1.11	1.06	1.13
1.13	1.59	1.54	1.56
1.14	1.60	1.55	1.66
1.15	1.61	1.56	1.68
2.25	2.95	2.92	3.04
2.36	3.09	3.06	2.84
2.38	3.11	3.09	3.17
2.66	3.45	3.43	3.41
2.77	3.59	3.57	3.35
2.80	3.62	3.61	3.70

<sup>1</sup> Equation No. 1 is the regression equation used in this study. Equation No. 2 is the regression equation of Binnington, Sibbitt, and Geddes (1938).

It will be seen that the values calculated from the two regression equations are virtually identical and are in excellent agreement with the

observed values. This agreement is especially good considering the fact that the equation derived in this study is calculated from data obtained in eight different laboratories and with three different methods of estimating pigment content. The standard errors of prediction are likewise essentially the same, being 0.14 ppm. for this study and 0.17 ppm. for the data reported by Binnington, Sibbitt, and Geddes.

To estimate the precision of the two techniques, the duplicate results were employed to calculate the standard error of a single determination which was found to be 0.026 ppm. for either or both solvents. Thus both solvents yield equally precise results. The butanol procedure, however, gives a better differentiation between samples, since the spread between values is greater with the same experimental error.

When comparisons are drawn between the various methods of estimating pigment content in extracts, the results are not so favorable. Inspection of the mean values for all flours presented in Table I indicates that the photoelectric procedure yields the highest results, and the visual technique the lowest. While these differences are not very great, the inclusion of results known to be of doubtful accuracy would change the picture considerably, and even employing only results of presumed reliability, a difference still exists between methods which is statistically significant. In this connection, the results obtained by two collaborators employing KWSZ photoelectric photometers are of interest and are presented in Table III.

TABLE III  
COMPARISON OF FLOUR CAROTENE VALUES, WATER SATURATED  
BUTANOL SOLVENT AND KWSZ PHOTOMETER

	Carotenoid pigment in flour, <i>ppm.</i>			
	A	B	Mean	General mean (Table I)
Flour				
A	0.36	0.47	0.42	0.82
B	0.64	1.96	0.85	1.56
C	0.41	0.58	0.50	1.02
D	1.11	2.18	1.65	2.92
E	1.27	2.69	1.98	3.40
F	1.27	2.65	1.96	3.45

It will be noted that the mean values are much lower than the general means in Table I, and also that poor agreement is shown between the two sets of results. Fundamentally there is no valid reason why such a discrepancy should exist, and it is believed that the explanation resides in the method of standardization employed. The significant differences between spectrophotometric, visual, and photoelectric results

are probably attributable to the same difficulty of standardization, and it would seem that this represents the major source of error in pigment determinations, since the precision of all three methods when calculated from the results of duplicate determinations is identical, the standard error of a single determination being again 0.026 ppm. in all cases.

Several of the collaborators commented favorably upon the rapid procedure, and six indicated that they are employing it as a routine test. Only one collaborator offered adverse criticism, and this referred only to the odor of the solvent, which he states necessitated working in a hood. Such practice does not appear to be general among those employing the method.

### Summary

The Binnington-Geddes rapid method for determining flour pigments has been subjected to collaborative study in the instance of six flours and nine collaborators.

Tests were made in comparison with the standard A. A. C. C. procedure employing naphtha + alcohol solvent. Various methods of estimating the pigment content of the extracts obtained were employed, including spectrophotometric, visual, and photoelectric procedures.

The rapid method extracts more pigment, thus yielding results of a higher order of magnitude than the naphtha + alcohol procedure. Regression equations are presented enabling conversion of results by one solvent into the other and *vice versa*. The error of prediction of butanol values from naphtha + alcohol is  $\pm 0.11$  ppm. These equations enable comparison of prior results with those obtained by the rapid procedure.

It is shown that both methods yield relatively similar results regardless of the method employed for estimating pigment content or of the laboratory in which the tests are performed. Both techniques show high and equal precision, the standard error of a single determination being 0.026 ppm. for either or both procedures. The rapid method gives a somewhat better differentiation between samples.

A statistically significant difference was found between the various methods of estimating pigment content, which may be primarily due to variations in the mode of standardization. It is suggested that methods of standardization and estimation be investigated with a view to increasing the accuracy of this phase of flour pigment determinations.

### Acknowledgments

The assistance of J. A. Anderson, Mark A. Barmore, W. F. Geddes, Willis S. Hutchinson, Harold Johansson, R. K. Larmour, V. W. Morris, A. G. O. Whiteside, and W. W. Worzella, who collaborated in this study, is gratefully acknowledged.

It is also desired to acknowledge the valuable assistance of Willis S. Hutchinson and R. I. Derby, who selected and prepared the samples and conducted the control storage tests, and of F. C. Hildebrand for assistance in statistical reduction of the data. This work was undertaken as a subproject of the Committee on Methods of Analysis.

#### Literature Cited

- American Association of Cereal Chemists  
1935 Cereal laboratory methods (3rd ed.). Published by the American Association of Cereal Chemists, New York, N. Y.
- Binnington, D. S., Sibbitt, L. D., and Geddes, W. F.  
1938 A comparative study of the utility of various commercially available organic solvents for the determination of wheat and flour pigments. *Cereal Chem.* 15: 119-132.
- Binnington, D. S., and Geddes, W. F.  
1939 A rapid method for the determination of wheat and flour pigments. *Cereal Chem.* 16: 252-262.
- Coleman, D. A., and Christie, A.  
1926 A rapid method for determining the gasoline color value of flour and wheat. *Cereal Chem.* 3: 84-89.
- Geddes, W. F., Binnington, D. S., and Whiteside, A. G. O.  
1934 A simplified method for the determination of carotene in flour extracts. *Cereal Chem.* 11: 1-24.
- Ferrari, C. G.  
1933 Spectrophotometric determination of the carotenoid pigment content of wheat flour. *Cereal Chem.* 10: 277-286.

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## COLLABORATIVE STUDY OF THE FERRICYANIDE METHOD FOR THE DETERMINATION OF DIASTATIC POWER OF MALT<sup>1</sup>

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(Read at the Annual Meeting, May 1940)

Last year's committee recommended that the activities for the present year be confined to a study of the determination of diastatic power. Previous work of the committee (Singruen, 1939) had showed that the ferricyanide method as modified by Anderson and Sallans (1937) gave less variation than the official method of the American Society of Brewing Chemists. Accordingly it was decided to carry out a comprehensive collaborative study of the ferricyanide method in the hope that statistical analysis of the data would give information on the variation in values obtained within any one laboratory and between laboratories.

Samples of three malts and a sample of soluble starch were sent to each of twelve collaborators with detailed directions for the procedure to be followed. Duplicate determinations were to be made on each malt on each of four days and all of the data reported, including standardizations of the solutions used.

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<sup>1</sup> Report of the Malt Analysis Standardization Committee (1939-1940).

<sup>2</sup> Stationed at Madison, Wisconsin.



TABLE I  
SUMMARY OF RESULTS ON COLLABORATIVE STUDY OF THE FERRICVANIDE METHOD FOR THE DETERMINATION  
OF DIASTATIC POWER OF MALTS—VALUES IN DEGREES LINTNER

	Collaborator											Mean collabo- rators	Range collabo- rators
	1	2	3	4	5	6	7	8	9	10	11		
Malt 1. Mean of 8 determinations	122.3	133.9	126.8	123.5	120.9	128.5	126.3	124.6	120.6	129.8	123.1	125.5	13.3
Range	3.8	3.4	3.2	4.8	4.5	4.3	3.0	1.9	1.6	2.3	3.1	3.3	—
Malt 2. Mean of 8 determinations	128.5	141.6	133.9	134.3	127.3	134.7	128.2	130.5	128.4	132.8	128.4	131.7	14.3
Range	5.1	4.3	4.7	13.7	10.4	5.4	6.9	2.0	3.9	1.8	4.3	5.7	—
Malt 3. Mean of 8 determinations	158.7	174.6	167.6	164.0	157.2	164.2	162.8	161.2	157.1	164.1	160.3	162.9	17.5
Range	8.3	5.4	5.0	14.4	18.7	5.4	9.0	5.1	4.6	2.8	6.5	7.7	—
Mean of malts	136.5	150.0	142.8	140.6	135.1	142.5	139.1	138.8	135.4	142.2	137.3	140.0	14.9
Mean range all malts	5.7	4.4	4.3	11.0	11.2	5.0	6.3	3.0	3.4	2.3	4.6	5.6	—
Range over days—mean all malts	3.1	2.1	1.0	9.0	6.1	4.4	3.3	0.8	1.4	0.8	1.7	3.1	—
Standard error of mean of dupli- cates, °L.	1.29	0.80	1.32	—	—	1.36	1.28	0.86	0.64	0.56	0.43	—	—

Results were submitted by eleven of the twelve collaborators, but the data from two laboratories reporting had to be omitted from the statistical analysis because some of the values requested were not obtained.

### Presentation and Discussion of Data

A summary of the data, giving the means for collaborators and the range in the values making up the means, is given in Table I. An inspection of these data shows considerable variation in the values obtained by the different laboratories and in the range within the eight single determinations in the individual laboratories. When the values of Collaborator 2, which are consistently high, are not considered, the other ten laboratories can be divided roughly into a high and a low group. The agreement in diastatic power within the two separate groups is very good. The variation between duplicate determinations, as indicated by the standard error of means of duplicates, also divides the collaborators into two groups, but the placement of the laboratories in the groups is not the same by the two methods. In other words there appears to be no consistent association of low duplicate errors with either low or high diastatic-power values. A test of significance was applied to the means of the two groups distinguished by the duplicate errors and it was found that they did not differ significantly.

TABLE II  
ANALYSES OF VARIANCE FOR COMBINED DATA

Variance due to	D.F.	Mean square
Samples	2	28,813.7**
Labs.	8	531.6**
Samples $\times$ Labs.	16	17.0

Note: In this and the following table, \*\* denotes that the 1% level, and \* that the 5% level of significance is attained.

As all the laboratories did not place the malts in exactly the same relative positions it was necessary to resort to statistical analysis in order to determine the significance of the differences between laboratories. In this analysis, only the mean diastatic-power values over all days for each laboratory were used. The variations within laboratories were treated separately. The results of the pooled analysis of variance are shown in Table II. These data show that, in comparison with the mean square due to the changes in the relative positions of the samples from laboratory to laboratory the differences between samples and the differences

between laboratories attain a highly significant level. The value necessary to show significant differences between laboratories is  $2.5^{\circ}$  L.

The results of the analyses of variance within laboratories are shown in Table III. In five of the nine laboratories the variations between

TABLE III  
ANALYSES OF VARIANCE FOR EACH LABORATORY

Laboratory	Mean squares			
	Samples 2 D.F.	Days 3 D.F.	S $\times$ D 6 D.F.	Duplicates 12 D.F.
9	2978.4**	2.3	2.4	0.9
1	3035.9**	12.1	3.6	3.3
11	3242.6**	3.8	8.9**	0.4
8	2965.7**	1.8	2.4	1.5
7	3375.2**	15.8	8.0	3.3
10	2893.8**	0.8	0.4	0.6
6	2921.0**	13.3*	0.9	3.6
3	3799.9**	1.5	1.9	3.5
2	3734.4**	6.0	3.4	1.3
Mean	3216.7	6.4	3.5	2.0

days are significantly greater than the variation between duplicates, but in only one laboratory (No. 6) is the effect of days significantly greater than the effect of interaction between samples and days. In one laboratory (No. 11) the variance due to interaction between malts and days is significantly greater than that due to differences between duplicates. Under ideal conditions the mean squares due to days, interaction between days and malts and duplicates should be of the same order and should be as small as possible. It therefore appears necessary for each laboratory to investigate its own sources of error.

The differences between laboratories in the diastatic-power values do not appear to be associated with any particular source of error within laboratories and the greatest error apparent in this test is the interaction between malts and laboratories. However, it is not unlikely that this error would be reduced in future tests conducted after the laboratories had investigated their own errors.

In two of the collaborating laboratories, different samples of Merck's Reagent Soluble Starch were used in addition to the sample sent out with the malts. In one laboratory significantly different values were obtained with the two starches, while in the second the differences between starch samples was not significant. This indicates that where two soluble starches differ sufficiently in their characteristics, their use will introduce an appreciable error in the results obtained. In collabo-

rative work on diastatic-power determinations a uniform sample of starch should be supplied to all collaborators.

The normality of the thiosulphate solutions was requested in this study and it afforded evidence of the magnitude of the error which might be ascribed to this one factor if the solutions were not standardized and the correction applied. The maximum variation between the reported values from ten laboratories was 0.0017, which if not corrected would be equivalent to approximately 4.0° Lintner based on the means of the three malts.

The unexplained variations in the values obtained in this collaborative study are still greater than desirable, but they are less than those obtained with any other method that has been studied collaboratively by this committee. Therefore the committee proposes the following recommendations:

That the ferricyanide method for the determination of diastatic power be adopted as tentative as well as the method of the American Society of Brewing Chemists (Coleman, 1936).

That certain phases of the method be studied further in an effort to reduce the variation and to standardize the procedure to a greater degree.

That the major emphasis of the committee be directed toward a study of the available methods for the determination of alpha-amylase, with the purpose of finding or developing an acceptable method.

#### Acknowledgments

The chairman wishes to express his appreciation to the members of the committee and to the collaborators for their excellent cooperation in this study and to acknowledge the assistance of W. O. S. Meredith in the statistical analysis and preparation of the report.

#### Literature Cited

- Anderson, J. A., and Sallans, H. R.  
1937 Determination of the diastatic power of malt in degrees Lintner by means of a ferricyanide reagent. *Can. J. Research* **C15**: 70-77.
- Coleman, D. A.  
1936 Report of the malt analysis standardization committee. *Cereal Chem.* **13**: 669-674.
- Singruen, E.  
1939 Report of the malt analysis standardization committee. *Cereal Chem.* **16**: 353-355.

# A COMPARISON BETWEEN THE OFFICIAL AIR-OVEN MOISTURE METHOD AND THE RAPID ALUMINUM-PLATE METHOD <sup>1</sup>

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(Read at the Annual Meeting, May 1940)

The aluminum-plate method used in the tests to be reported was that described by Sandstedt.<sup>2</sup> The official method is that which is given in the A. A. C. C. book of methods. The official method was interpreted to mean one hour of total time for the sample in the oven. This interpretation was used for two reasons: first, it is the interpretation which was used in the development of the rapid method and second, it is the interpretation placed upon the method by a majority of control chemists using the procedure.

The problem of comparing the two methods was approached with the idea of determining the effects of position of the sample in the oven and spreads within the methods as well as between the methods.

Every possible precaution was taken to eliminate errors in sampling and the sequence of weighing of each sample was recorded. Three flour samples were used and the results obtained are summarized in Table I.

TABLE I  
COMPARISON OF OFFICIAL AND ALUMINUM PLATE METHODS WITH FLOUR SAMPLES

Sample number	Official method			Aluminum plate method		
	Number of deter- minations	Mean	Range	Number of deter- minations	Mean	Range
		%	%		%	%
1	10	14.36	0.40	10	14.42	0.20
2	10	10.69	0.25	10	10.69	0.10
3	32	10.63	0.30	32	10.62	0.20

Two samples of ground wheat were used for a comparison of the methods on this material. Precautions were again taken to insure freedom from sampling differences. The time for the aluminum-plate method was varied from 15 to 40 minutes. The results obtained are summarized in Table II.

The writer believes that the average operator fails to recognize the significance and magnitude of errors that are obtained as a result of

<sup>1</sup> Journal Series No. 269, Nebraska Agricultural Experiment Station.

<sup>2</sup> R. M. Sandstedt, The rapid determination of moisture, Cereal Chem. 15: 813-815, 1938.

TABLE II  
COMPARISON OF OFFICIAL AND ALUMINUM PLATE METHODS  
WITH GROUND WHEAT SAMPLES

Number Sample of deter- minations		Mean	Range	Time	Number of deter- minations	Mean	Range
		%	%	Min.		%	%
1	20	17.57	0.50	15	10	17.18	0.40
				20	20	17.40	0.35
				25	10	17.38	0.20
				40	10	17.68	0.30
2	15	16.02	0.25	20	15	15.84	0.35
				40	15	16.06	0.25

loss of moisture while weighing a series of samples from the same container. Errors greater than those inherent in the method may be obtained because of losses of moisture during low humidity periods. This is perhaps particularly true of the Southwest where the relative humidity in the laboratory is abnormally low during the winter months. How-

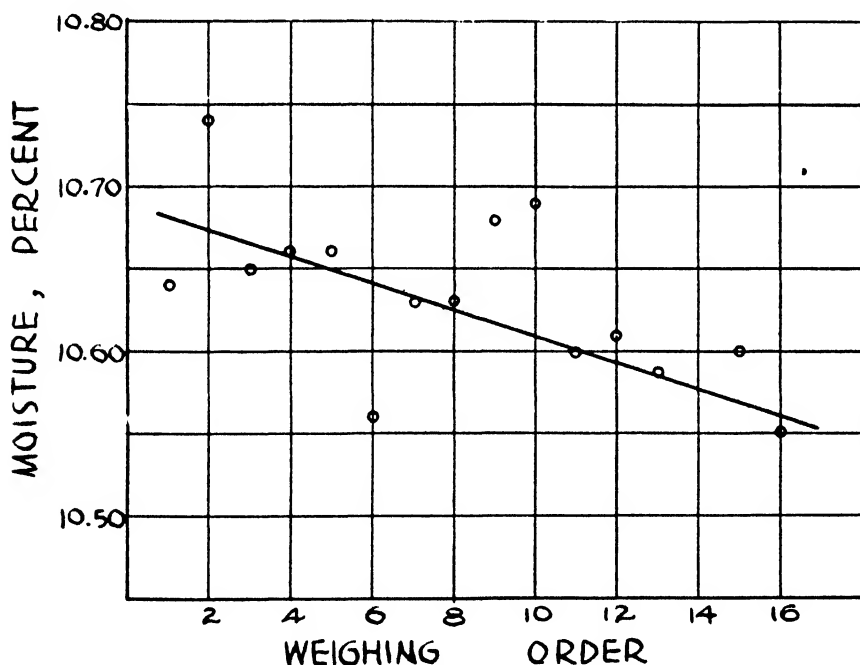


Fig. 1. The relation between moisture loss and the number of samples weighed from a container.

ever, even in other parts of the country where low temperatures prevail, air contains very little moisture after being raised from outdoor temperatures to normal room temperatures. With this thought in mind the average results obtained from samples weighed in sequence of one to sixteen were plotted against the position in the sequence. The results are represented graphically in Figure 1.

### Conclusions

The two methods when used on flour samples gave results which are in agreement well within the limits of error of either method.

There is a lesser tendency for variation caused by position in the oven when the rapid aluminum plate method is used than where the air oven is used. Care must be taken not to place samples too near the front of the oven when the official air method is used.

The rapid method does not give satisfactory results on samples of ground wheat. Preliminary indications are that ground wheat samples must be heated for a period of time closer to 40 minutes than the formerly recommended 20 minutes if comparable results are to be obtained.

### Recommendations

It is recommended that the rapid method be considered comparable to the official method (if the above interpretation of the air-oven method is held to be correct) and that further work be done on the determination of moisture on ground wheat samples. It is entirely possible that the use of a smaller sample would allow the determination to be made with comparable results in as short a period of time as that used for flour samples.

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# DETERMINATION OF MOISTURE IN FLOUR AND GROUND WHEAT—A COLLABORATIVE COMPARISON OF THE OFFICIAL AIR-OVEN AND RAPID ALUMINUM-PLATE METHODS<sup>1</sup>

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(Read at the Annual Meeting, May 1940)

In accordance with the recommendation of the 1938-39 Committee on Methods of Analysis, the comparison between the rapid aluminum-plate method for determining moisture in flour and wheat (Sandstedt, 1938) and the "official" air-oven method (drying for one hour at 130°C., was continued. Previous work suggested that agreement within practical limits was possible when flour was dried for 15 minutes on the aluminum plate. Results with ground wheat dried for 20 minutes did not agree with those obtained in the air oven.

Twenty-six collaborators were enlisted whose laboratories were equipped with standard makes of air ovens and the necessary cast or cold rolled aluminum plates one-half inch or more in thickness. To reduce variables, specific directions were given for standardizing equipment and handling samples. Four 2,000-gram subsamples were weighed from each. The first and third subsamples were dried by the air-oven method, the second and fourth by the rapid aluminum-plate method. Additional 2,000-gram samples were supplied as needed to make each oven load consist of eight samples, on the assumption that the objective of speed is defeated when a larger number of samples are accumulated before drying is started.

Collaborators observed the following general precautions: to have open all oven vents, to avoid large temperature drops by loading the oven quickly, to avoid placing samples under the pilot light, to place covers atilt on their respective dishes during drying, to replace covers promptly while dishes were still in the oven, to cool dishes in a desiccator, and to reweigh them soon after they reached room temperature (allowing not more than 20 minutes for cooling after taking dishes from the drying oven).

For making the "official" air-oven tests, the ovens were regulated to give a temperature of 130°C. (plus or minus 3°) measured by a thermometer having the tip of the bulb level with the top of the moisture dishes but not directly over a sample. Neither an aluminum plate nor a forced draft was used. Collaborators dried the dishes, covers, and con-

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<sup>1</sup> Subcommittee report of the 1939-40 Committee on Methods of Analysis.



tents for one hour, beginning when the temperature of the oven regained 127°C.

The instructions applying specifically to the aluminum-plate method were: to place the half-inch (or more) thick aluminum plate on the lowest shelf support, to regulate the oven so that the temperature of the aluminum plate was 140°C. (plus or minus 1°) measured by a thermometer (the bulb of which rested on the bottom of a small can containing fine sand), and to dry the dishes, covers, and contents for a total of 15 minutes for flour and 20 minutes for ground wheat.

The first, middle, and last individual samples drawn from each general sample were retained and analyzed in duplicate in our laboratory on the day specified to the collaborators for their tests. These six results on each general sample were in excellent agreement, and reflected the great care used in preparing the samples.

Table I shows the moisture range of the seven flours used and the apparent close agreement between the averages obtained by the two methods.

TABLE I  
AVERAGES OF MOISTURE DETERMINATIONS BY EACH METHOD ON FLOUR

Sample No.	(1) AOAC av.	(2) A. P. av.	Deviation of (2) from (1)
5	9.71	9.66	-.05
6	12.61	12.53	-.08
7	12.13	12.08	-.05
8	12.10	12.04	-.06
9	13.06	12.99	-.07
10	8.95	8.92	-.03
11	11.74	11.70	-.04

Samples 5 to 8 were soft-wheat flours; 9 to 11, hard-wheat flours. It may be observed that while the differences between the averages are small (being from 0.03% to 0.08% moisture), all by the aluminum-plate method are lower than by the air-oven method.

In Table II similar data are given for six samples of ground wheat. Differences between corresponding averages, all negative, are larger than those for flour in spite of the 20 minutes' drying on the aluminum plate.

The individual results obtained by all collaborators on all samples are summarized in Table III, which shows, in increments of one-tenth percent, the deviation of results by the rapid aluminum-plate method from the corresponding 130°C. air-oven results. The averages of duplicates in 96 comparative tests on soft-wheat flours showed an average

TABLE II  
AVERAGES OF MOISTURE DETERMINATIONS BY EACH METHOD  
ON GROUND WHEAT

Sample No.	(1) AOAC av.	(2) A. P. av.	Deviation of (2) from (1)
12	8.66	8.57	-.09
13	8.82	8.71	-.11
14	6.38	6.19	-.19
15	8.64	8.50	-.14
16	13.71	13.58	-.13
17	6.30	6.12	-.18

TABLE III  
SHOWING PERCENT OF TESTS DEVIATING FROM 130°C. AIR-OVEN RESULTS

Increments 0.1% each	Soft- wheat flour	Hard- wheat flour	Ground wheat
0.0 to 0.1	54.2	56.8	29.8
0.1 to 0.2	31.3	18.9	27.5
0.2 to 0.3	10.4	13.5	20.7
0.3 to 0.4	3.1	6.8	13.0
Over 0.4	1.0	4.0	9.0
Av. dev.	0.116	0.134	0.204
No. tests	96	74	131

deviation of less than 0.12% of moisture. Of these 54.2% deviated by 0.10% or less and a total of 85.5% by 0.20% or less from the 130°C. air-oven method. The averages of duplicates in 74 comparative tests on hard-wheat flours showed an average deviation of less than 0.14% moisture. Of these 58.6% deviated by 0.10% or less, and a total of 75.5% by 0.20% or less, from the 130°C. air-oven method.

The data shown in Table III are displayed in a different form in Table IV to emphasize that although a majority of the aluminum-plate-

TABLE IV  
SHOWING PERCENT OF TESTS DEVIATING FROM 130°C. AIR-OVEN RESULTS

Increments 0.1% each	Soft- wheat flour	Hard- wheat flour	Ground wheat
-0.4 or less	3.1	8.1	17.5
-0.3	10.4	10.8	19.1
-0.2	21.9	12.2	20.6
-0.1	20.8	27.0	16.8
0.0	6.3	5.4	2.3
0.1	27.1	24.3	10.7
0.2	9.4	6.8	6.9
0.3 or more	1.0	5.4	6.1

method results on flour agree with the air-oven method within 0.20%, there were more negative than positive deviations, as was indicated by the small average negative differences reported in Table I. Specifically, Table IV shows that 6.3% of the corresponding determinations on soft-wheat flour were in perfect agreement, whereas 42.7% had a negative deviation of less than 0.20% moisture contrasted with the 36.5% which had a positive deviation of up to 0.20% moisture, showing a total of 85.5% within plus or minus 0.20%. Also, it shows for the hard-wheat flours that 5.4% of the corresponding determinations were in perfect agreement, whereas 39.2% had a negative deviation of less than 0.20% moisture, while 31.1% had a positive deviation up to 0.20% moisture, giving a total of 75.7% within plus or minus 0.20%.

The principal disadvantage of the aluminum-plate method as now specified appears to be the difficulty of accurately measuring the temperature of the aluminum plate itself. Several collaborators have proposed replacing the can of sand surrounding the thermometer bulb by either a mercury well or a can containing fine aluminum shot. This should be tried as it probably would improve agreement between collaborators.

As reported by Sandstedt (1938) one of the chief advantages of using the aluminum plate is the even distribution of heat over the entire shelf area which in effect increases the useful capacity of any drying oven (air or vacuum) because even the spaces near the door and under the pilot lamp can be used. When the aluminum plate is used small differences from the specified temperature do not affect seriously the accuracy of results because rate of heat transfer to the samples remains high.

The fact that 85.5% of the soft-wheat-flour results and 75.7% of the hard-wheat agree within 0.20% (as between the two methods) indicates that the aluminum-plate method can be made to function well enough to be used for mill control testing. In any one laboratory, these variations can be reduced by careful calibration with respect to the official method. The experimental error between collaborators using either method in this study was found to be not significantly greater than that reported by Davis and Wise (1933) in their analysis of the systematic error experienced in collaborative work by members of the Pioneer Section.

Therefore we recommend the rapid aluminum-plate method, 140°C. for 15 minutes, as an alternative method for the rapid determination of moisture in flour, and on this basis we suggest that no further collaborative comparisons be made on flour. The results with ground wheat are not satisfactory. As shown in Table III, only 29.8% of the results

agree within 0.10% and only 57.3% within 0.20%. The average deviation of the 131 comparisons is slightly more than 0.20% moisture. In Table IV, 74.0% of the results on ground wheat are seen to have a negative deviation. However, application of the rapid method to ground wheat cannot yet be condemned because it probably can be made to give results equivalent to those obtained by the official 130°C. one-hour method. Some may question the use of the 130°C. one-hour drying method for moisture in ground wheat. While no specific references appear in either the A. O. A. C. or the A. A. C. C. books of methods, the U. S. Department of Agriculture, Bureau of Agricultural Economics, issued a Service and Regulatory Announcement No. 147, April, 1935, describing an approved procedure for making these tests on ground wheat, and the 1934-35 A. A. C. C. Committee on Methods of Analysis recommended "that the vacuum-oven and air-oven (130°C. for 1 hour) methods be continued as the official methods for wheat feeds as well as flour."

Assuming the air-oven method as a reference point in testing wheat, we see no difficulty in further modifying the proposed 140°C. aluminum-plate method by increasing the drying time until equivalent results are obtained. Therefore we recommend that further work be done on ground wheat in a designated laboratory to determine new specifications to be subjected later to collaborative study.

It should always be borne in mind that the literature contains many references to probable sources of error in moisture testing. Bailey (1914) discussed the "non-uniformity of drying oven temperatures." Lanning (1935) showed that a poorly designed thermostat contributed to uneven temperatures in a small drying oven. He recommended defining the position of the thermometer bulb in relation to the samples and the time to be allowed for the oven to regain 130°C. Treloar and Sullivan (1935) in studying the variability of data obtained on feeds by the vacuum oven, air oven at 130°C. for one hour, and air oven at 135°C. for two hours, concluded that "there is no demonstrable gain in consistency through using the higher temperature and longer time in the air oven." Davis (1935) found that different oven ventilation conditions had little effect on the results by the air-oven method, 130°C. for one hour.

In view of these many papers and the commonplaceness of the moisture test, it may appear superfluous repetition to say that the fundamentals of what is essentially an empirical, physical test frequently are ignored. Therefore, by way of emphasis, we recommend to the Committee on Revision of Cereal Laboratory Methods:

1. Clarification of the language of the method to show that: "The one-hour drying period begins when the temperature of the oven regains 130°C." (This statement, according to Dr. Henry A. Lepper, editor, will appear in the forthcoming edition of *Methods of Analysis*, A. O. A. C.)

2. A statement specifying where the tip of the thermometer bulb shall be placed in relation to the samples being dried.

### Acknowledgment

We wish to acknowledge the assistance given by our collaborators, without whose careful work and helpful comments this report would have been impossible.

### Literature Cited

- Bailey, L. H.  
1914 Non-uniformity of drying oven temperature. *J. Ind. Eng. Chem.* **6**: 585.
- Davis, Claude F.  
1935 Forced draft as an aid in air-oven moisture tests. *Cereal Chem.* **12**: 512-517.
- Davis, C. F., and Wise, M.  
1933 A five-year summary of the monthly check sample reports of the Pioneer Section of the A. A. C. C. *Cereal Chem.* **10**: 203-212.
- Lanning, J. H.  
1935 A study of temperature changes in a small laboratory oven when drying flour by the 130°C. oven method. *Cereal Chem.* **12**: 69-77.
- Putnam, H. W.  
1938 Collaborative study of the 15-minute moisture method in comparison with the official air-oven method. *Cereal Chem.* **15**: 816-818.
- Sandstedt, R. M.  
1938 The rapid determination of moisture. *Cereal Chem.* **15**: 813.
- Sullivan, B.  
1935 Report of the Committee on Methods of Analysis. *Cereal Chem.* **12**: 505.
- Treloar, Alan E., and Sullivan, B.  
1935 The determination of moisture in feeds. A comparison of three moisture methods. *Cereal Chem.* **12**: 520-527.

# NOTE ON MODIFICATION OF THE A. A. C. C. METHODS FOR THE DETERMINATION OF TOTAL AND RESIDUAL CO<sub>2</sub> IN BAKING POWDERS AND TOTAL CO<sub>2</sub> IN SELF-RISING FLOURS

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(Read at the Annual Meeting, May 1940)

This note is presented with the thought that the modifications as used in our laboratories in the routine testing of CO<sub>2</sub> in baking powders and self-rising flours may help other investigators to secure reproducible results and may also save considerable time.

Determination of the residual CO<sub>2</sub> in baking powders<sup>1</sup> involves a step wherein a rather thick, gelatinous suspension is boiled for one minute. We have found that often there is insufficient water present to permit the starch to become dextrinized and caramelized, in which case high CO<sub>2</sub> values are obtained. A simple preventive measure is the addition of 2 to 3 cc. of water just before boiling. A heating period slightly longer than one minute is not necessary. As much as 5 cc. of added water has been found to have no adverse effect on the recovery of CO<sub>2</sub>.

A modification has been used also in the gasometric measurement of residual and total CO<sub>2</sub> in baking powders and of CO<sub>2</sub> in self-rising flours. Methods for total CO<sub>2</sub> include a step in which from 5 to 10 minutes of standing is required after the CO<sub>2</sub> has been liberated and before the final reading of the gas burette. This is to insure equalization of the temperature of the gases in the flask and the burette. A simple way to reduce the time for each determination is to use acid which has been adjusted in temperature to about 1°C. below room temperature when testing for residual or total CO<sub>2</sub> in baking powders and about 2°C. when testing self-rising flours for total CO<sub>2</sub>. A thermometer inserted through a third hole in the stopper holding the CO<sub>2</sub> flask will indicate the temperature of the suspension. Acid sufficiently below room temperature is used so that after shaking for the prescribed time, the temperature within the flask will have attained that of the gas burette, *i.e.*, room temperature. Gas volume may then be recorded immediately with little loss of time. It has been observed that upon different days slightly different temperature adjustments of the acid are required.

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<sup>1</sup> Cereal Laboratory Methods, Chapter 9, Methods 2 and 3, pp. 114-117, 1935.





# CEREAL CHEMISTRY

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VOL. XVII

NOVEMBER, 1940

No. 6

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## THE FUNCTION OF THE LIPIDS IN MILLING AND BAKING<sup>1</sup>

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(Received for publication May 10, 1940)

Most of the research on wheat and flour has been centered on the proteins in as much as the quality of gluten (the characteristic protein of wheat) has seemed in large measure to determine the desirability of any given flour for bread-making purposes. Until recently little work had been done on the lipids and their influence in milling and baking. Our knowledge on this subject is still woefully incomplete.

Milling itself is an entirely mechanical procedure and the lipids do not affect the actual mechanical operations of breaking, sifting, and air purification except insofar as they alter the physical characteristics such as friability and specific gravity of the various parts of the wheat kernel.

The ideal in milling is the complete removal of both the bran and the embryo from the endosperm of the wheat because, by themselves, neither bran, the outer envelope of the kernel, nor germ is capable of making a loaf of bread when mixed with water, yeast, and salt and handled in the usual manner. Neither bran nor germ can retain the carbon dioxide gas developed in fermentation to produce the well-risen, palatable bread which can be made with white flour.

Another reason why the miller attempts as complete a removal as possible of the bran and germ is that these parts of the berry decrease the keeping quality of flour, owing, in part, to the proportionally higher fat content of the bran and germ as compared to the endosperm.

The higher lipid content of the germ, in particular, makes its physical character different enough to allow an easy, though incomplete, separation from the flour.

The distribution of the lipids in the various separations made on a modern mill from hard spring wheat is as follows.

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<sup>1</sup> Paper presented at the Cincinnati meeting of the American Chemical Society, April 8-12, 1940.



	Percent Lipids (Alcohol-ether extract) (Calculated to 13.5% moisture)
Wheat	3.0
Short patent	1.3
Straight	1.6
Clear	2.7
Low grade	3.2
Bran	6.5
Shorts	7.5
Germ	15.5

These figures are only approximate and will, of course, vary with the type of wheat, the percentage extraction, and the method of milling.

The better grades of flour, from both a storage and a baking standpoint, have the least possible amount of bran and germ and, therefore, a rather low lipid content. Even though low, the lipids of flour are of considerable importance.

### Lipids of Wheat Products

Wheat germ makes up about 2% of the weight of the wheat kernel. An average separation contains approximately 15% of lipids of which 4% to 10% is considered as the phosphatide fraction. The work of Channon and Foster (1934) has shown that the phosphatide fraction of germ is made up of phosphatidic acid (as Ca, Mg, and K salts), lecithin, and cephalin. These compounds occur, according to Channon and Foster, in the proportion of 4:4:1 when referred to the phosphorus content.

The unsaponifiable material from an average germ separation amounts to 4% to 5% of the oil, of which about 70% is a mixture of the saturated sterol, dihydrositosterol, and several doubly unsaturated sterols called  $\alpha_1$ ,  $\alpha_2$ ,  $\alpha_3$ ,  $\beta$  and  $\gamma$  sitosterol (Anderson, Shriner and Burr, 1926; Wallis and Fernholz, 1936; and Bernstein and Wallis, 1939). The material remaining after the separation of the sterols is a yellow oil containing among other polyenes the tocopherols which possess vitamin E activity and certain of which have been recently synthesized.

The fatty acids obtained after the removal of the unsaponifiable material from wheat germ have been identified as follows (Sullivan and Bailey, 1936):

Palmitic acid	11.76
Stearic acid	3.06
Lignoceric acid	1.18
$\alpha$ linolenic acid	1.83
$\beta$ linolenic acid	1.72
$\alpha$ linolic acid	22.32
$\beta$ linolic acid	29.99
Oleic acid	28.14

The saturated acid fraction amounting to 16% was composed largely of palmitic acid, while the unsaturated fraction contained over 52% of linolic acid.

The lipids of wheat flour contain more volatile fatty acids than those of wheat germ. As is the case with the wheat-germ lipids, the bulk of the saturated fraction is palmitic acid. The same unsaturated acids, oleic, linolic, and a small amount of linolenic are present, as in wheat germ, with linolic the predominating acid of the unsaturated series. Flour lipid contains a somewhat higher amount (5.5%) of unsaponifiable material than wheat-germ lipid with, however, only about one-half instead of 70% of the unsaponifiable fraction being precipitated by digitonin (Sullivan and Howe, 1938). Other separations of flour and feed do not vary widely in the distribution of the fatty acids present in the glycerides and vary only from 4% to 7% in the amount of unsaponifiable material.

Wheat, as is true of so many other plant products, has a certain amount of its total lipids bound with the proteins and carbohydrates in complexes such that the usual fat solvents, *i.e.* ether, acetone, and ethyl acetate, do not give complete extraction. Hence, to obtain the phosphatides and other fat-like compounds as well as the glycerides, a preliminary treatment with alcohol or ammonia must be used previous to extraction by ether. The difficulty in the removal and purification of plant phosphatides is well known to all workers in this field. It has been found extremely difficult to purify the phosphatides from either flour or the embryo. Practically all preparations contain some sugar (approximately 2%) even after repeated precipitations with acetone. With most milling separations the N:P ratio is always in excess of 1. The phosphatide preparations from wheat germ, however, show a N:P ratio of nearly 1:1 according to our results. Because of the high ratio of N:P (7:1) in the phosphatide separation from flour it is evident that there is either protein or some nitrogen-containing lipids other than lecithin or cephalin. Work in this laboratory, which we hope to publish shortly, has shown that the lipids from flour carry a nitrogen-containing substance with an  $S-S \rightleftharpoons S-H$  linkage and that this compound can be concentrated in the acetone insoluble fraction of the fat. The lipids from the embryo do not give the test for the S-S or S-H linkage. The compound in question has not as yet been obtained in a sufficient state of purity to be identified. Balls and Hale (1940) have recently published a preliminary report on this substance.

### The Influence of the Lipids on the Keeping Quality of Wheat Products

Flour, especially low-grade flour, bran, and germ contain lipases which under proper conditions of moisture, temperature, and pH hydrolyze the glycerides forming fatty acids (Sullivan and Howe, 1933). The hydrolysis is much more rapid at the higher moisture and temperature levels. If flour is stored in a cool dry place it maintains its baking quality for several months. Storage for long periods, especially in warm humid climates, causes the flour to deteriorate and become unsatisfactory for bread-making purposes. Such a damaged flour exhibits a "short" gluten and dough—that is, a dough that lacks extensibility and tears easily. The handling characteristics of the dough, its gas retention, volume, flavor, and taste, all suffer considerably in bread made from such a flour.

A flour which has gone out of condition for this reason may be extracted with ethyl ether, the fatty acids and fat removed, and fresh-flour fat (extracted from fresh flour of the same grade with ether) added back in the amount originally present in flour with the result that the baking quality becomes normal. The ether-extracted, aged flour without the addition of the ether extract from a fresh flour gives poorer volume in many instances than the out-of-condition flour. It is interesting in this connection that although fat from a freshly milled flour will bring back to normal the ether-extracted sample, fat from fresh-wheat germ will not, although the latter improves the handling characteristics of the dough. Since the fatty acids of the glycerides are not significantly different in kind and amount in any of the various milling separations we have not as yet succeeded in explaining this observation.

Kosmin (1934, 1934a, 1935) has shown that unsaturated fatty acids influence the colloidal behavior of the gluten by thickening the gel. Saturated acids show no such effect. Resnitschenko and Popzowa (1934), in an extension of Kosmin's work found that the effect of unsaturated acids on gluten was not due to their acidity as such but to the presence of the group  $RCH:CHCOOM$  where  $R$  = radical and  $M$  a metal or hydrogen. Both Kosmin and Resnitschenko and Popzowa judged the action of these compounds only by the feel of the hand-washed gluten.

Our own work (Sullivan, Near, and Foley, 1936) has shown that the unsaponifiable material from the lipids of the various separations and the simple triglycerides such as tripalmitin, tristearin, trimyristin, and triolein have little or no effect on the behavior of a patent flour as

shown by plasticity measurements and baking tests. The higher saturated fatty acids which may occur in small amounts in any wheat product as a result of hydrolysis of the triglycerides have only a very slight "shortening" effect on the flour. Unsaturated fatty acids such as oleic, linolic, and linolenic produce "short," tough, and brittle glutens when added to sound normal flour. These unsaturated acids, however, are only slightly detrimental to the baking quality of flour. The bread is not harmed as much as one would expect from the feeling of the gluten. Individual unsaturated fatty acids influence a flour in direct correlation with their increasing number of double bonds. Unsaturated fatty acids when exposed to oxygen have a marked damaging action on the baking quality of flour. The dough feels dead, loses its elasticity and breaks easily. The volume of the loaf is decreased considerably. The action of these oxidized acids when added to fresh flour is identical with that of extreme natural aging of flour. The unsaturated acids which result by progressive hydrolysis of glycerides and phosphatides on aging a flour do not do much harm to the baking quality until they become oxidized either by oxidases present in flour or by the oxygen of the air. The oxidized products are peroxides rather than hydroxy acids.

Sinclair and McCalla (1937) found that the volume of an aged, badly deteriorated flour could be brought back by 5% of fresh germ but not by the alcohol-ether extract of the germ. These investigators thought that this effect might be due to the more insoluble lipids of the germ. It is our belief that the beneficial effect of this fresh germ on the aged flour sample was due to the presence of glutathione which has been found in the fresh germ and which reduces the oxidized fat.

The byproducts of milling—bran, middlings, and germ—are subject to a similar hydrolysis of the lipids. On aging, particularly in a sealed closed container, the alcohol-ether extract decreases, the ether extract increases and the phosphorus and nitrogen contents of the extracts decrease. The changes in amount of total lipid (alcohol-ether extract), in acidity, and in phosphorus and nitrogen content of the extracts are very much less marked when the samples are stored at 4% moisture than when stored at 13% or above. The higher the moisture content the greater the enzymic action, as might be expected. Samples of wheat or its products which are stored in cotton bags show relatively less change in all of the above factors (Sullivan and Near, 1933).

Thus it can be seen that the more complete the removal of the bran, shorts, and germ (all of which are higher than the flour in lipids and enzymic activity), the less serious are the storage and transportation problems.

### The Influence of the Lipids on the Baking Quality of Flour

As has already been mentioned, a flour whose fat has become oxidized will show poor baking characteristics as reflected in lowered elasticity of the dough, as well as in poorer volume, texture, and grain of the baked bread.

A few workers have found that the ether extraction of a flour improves it while others working on different types of flour have found a decided detrimental effect. It has been our experience that ether extraction injures the breadmaking value of a flour although the gluten is in no way harmed. The dough made from the extracted sample is much tougher and more rubbery as is also the crumb of the finished bread. The volume of the loaf is considerably lowered. These characteristics may be restored completely by adding the extracted fat to the ingredients used in baking. Germ fat, cottonseed oil, lard, and many other shortenings will not fully bring back the extracted flour. We have found nothing so far which equals the flour fat in bringing back to normal an extracted flour.

Lecithin is beneficial in giving a smoother feeling dough but otherwise, when added in small amounts to a sound normal flour, it has very little effect on the baking properties. Shortenings such as lard, butter, or hydrogenated vegetable fat are usually added to a bread dough in amounts of from 2% to 4%. Shortening enables the sponge to be broken up more easily, gives a smoother dough and a more tender crumb. However, satisfactory though not quite as good bread can be made without shortening. Baker and Mize (1939) have shown that doughs which contain no shortening or liquid fats may become porous, allowing the expanding gas to escape, while dough containing semisolid shortening can retain much of the gas until after starch swelling and gluten coagulation in the oven. The phosphatides as well as other lipids *naturally* present in flour present a special problem.

When gluten, consisting principally of gliadin and glutenin, is washed out from flour it contains over half of the lipids of the flour in intimate combination. The gluten must first be treated with alcohol in order to liberate the lipids since only a trace can be removed by direct extraction with ethyl ether or petroleum ether. From 5% to 10% of the weight of gluten is lipid material. Gluten owes its singular properties in part to the lipids which are either adsorbed or in complex formation with the proteins.

Dough, which is an extremely complex colloidal system, has been thought of as a meshwork of coiled protein fibrils with starch and water. Lipids because of their property of reducing surface tension are con-

sidered to be distributed at the interfaces of this meshwork (Working, 1924). Too close an adhesion of the protein strands is prevented by the lipids which give more slippage and greater extensibility to the dough.

Bungenberg de Jong (1938) has shown that a pronounced shifting of the maximum to the acid side in the turbidity pH curve of an alkaline gluten sol occurs on the addition of small amounts of lecithin.

Toughness of a flour is in some way associated with the lipids. While there is much too little known concerning the exact role of the lipids in determining dough characteristics, all of the available findings indicate that the lipids are an extremely important factor in the proper estimation and knowledge of gluten quality. It is upon the amount and characteristics of the gluten that the baking quality, in a large measure, depends.

It is to be hoped that future work will throw more light on this problem.

#### Literature Cited

- Anderson, R. J., Shriner, R. L., and Burr, G. O.  
1926 The phytosterols of wheat germ oil. *J. Am. Chem. Soc.*, **48**: 2987-2996.
- Baker, J. C., and Mize, M. D.  
1939 Effect of temperature on dough properties. II. *Cereal Chem.* **16**: 682-695.
- Balls, A. K., and Hale, W. S.  
1940 A sulfur-bearing constituent of the petroleum ether extract of wheat flour. *Cereal Chem.* **17**: 243-245.
- Bernstein, S., and Wallis, E. S.  
1939 Studies in the sitosterol complex. The isolation of  $\alpha$ , sitosterol. *J. Am. Chem. Soc.* **61**: 1903-1904.
- Bungenberg de Jong, H. L.  
1938 Proteins in the flour industry. Symposium on Proteins. *Nederlandsche Chemische Vereeniging, Eindhoven*, p. 160.
- Channon, H. J., and Foster, C. H. M.  
1934 The phosphatides of wheat germ. *Biochem. J.* **28**: 853-864.
- Kozmin, N.  
1934 Reifung der Weizenmehle und ihre biochemischen Grundlagen. *Mühlenlab.* **4**: 18-31.  
1934a Kleberqualität und einige Faktoren, die diese beeinflussen. *Mühlenlab.* **4**: 109-116.  
1935 The aging of wheat flour and the nature of this process. *Cereal Chem.* **12**: 165-171.
- Resnitschenko, M. S., and Popzowa, A. Q.  
1934 Über die Wirkung ungesättigter Fettsäuren auf die Kolloide Beschaffenheit des Klebers. *Mühlenlab.* **4**: 57-62.
- Sinclair, A. T., and McCalla, A. G.  
1937 The influence of lipoids on the quality and keeping properties of flour. *Chem. J. Research* **C15**: 187-203.
- Sullivan, B., and Bailey, C. H.  
1936 The lipids of the wheat embryo. I. The fatty acids. *J. Amer. Chem. Soc.* **58**: 383-390. II. The unsaponifiable fraction. *J. Am. Chem. Soc.* **58**: 390-393.
- and Howe, M.  
1933 Lipases of wheat. I. *J. Am. Chem. Soc.* **55**: 320-324.  
1938 Lipids of wheat flour. I. The petroleum ether extract. *Cereal Chem.* **15**: 716-720.

- and Near, Cleo  
1933 Change of crude lipides of wheat on storage. *J. Ind. Eng. Chem.* **25**: 100-103.
- , Near, Cleo, and Foley, Guy H.  
1936 The role of the lipids in relation to flour quality. *Cereal Chem.* **13**: 318-331.
- Wallis, E. S., and Fernholz, E.  
1936  $\alpha$ -sitosterol. *J. Am. Chem. Soc.* **58**: 2446-2448.
- Working, E. B.  
1924 Lipoids, a factor influencing gluten quality. *Cereal Chem.* **1**: 153-158.

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## THE EFFECT ON DIASTATIC ACTIVITY OF SURFACE, PRESSURE, DIFFERENTIAL, AND TEMPERATURE OF THE REDUCTION ROLLS IN MILLING

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(Read at the Annual Meeting, May 1940)

A great effort has been made during the last twenty years to standardize analytical and baking methods for testing wheat. There is a corresponding need with reference to the experimental milling test, which is to-day often carried out exactly as it was thirty years ago, when Willard and Swanson (1911) published the details of their method and equipment. The necessity was recognized by the Milling and Baking Section of the Sixth International Technical and Chemical Congress of Agricultural Industries held in Budapest (Hungary) in July, 1939. This section passed a resolution that the laboratory milling test should receive priority in discussions at the next congress to be held in Rome.

### Factors Involved in Milling

A few of the following isolated factors have already been studied by various workers. They will probably have to be tested in conjunction with other factors, as there is a lot of overlapping in their effects.

I. *Break rolls*: diameter, corrugation (number and form), surface, degree of spirality, differential, speed, length of roll.

II. *Smooth rolls*: diameter, surface (hardness of material, microscopic structure), true running, differential, speed, pressure, temperature, length of roll.

III. *Product*: hardness (type of wheat, preparation), moisture, fineness or granulation, characteristics of starch (resistance to mechanical treatment), quality of gluten (resistance to mechanical treatment), temperature.

IV. *Air in milling room or mill*: temperature, relative humidity.

V. *Milling technique*: flow sheet, roll adjustment, sifting and purification.

The whole problem is complicated by the fact that the effects on diastatic activity and on gluten quality are often diametrically opposed to one another, as has been mentioned by Gründer (1935), Pulkki (1938), and others.

It is surprising that so little is to be found on the subject of the present paper, even in well-known milling books, such as those by Kosmin (1921), Kettenbach (1922), Miller (1923), Dedrick (1924), and Hopf (1938). Most publications have been concerned with the influence of smooth rolls on the granulation of flour, a subject which has recently been reviewed by Kent-Jones (1939).

Through different methods of casting, very different surface structures of milling rolls can be obtained. About the only published reference to this is that of Raym and Scharffenberg (1932),<sup>1</sup> and it is also mentioned by Hopf (1938). Geddes and Frisell (1935) used steel reduction rolls which had been hardened by carbonizing.

No reference was found to the actual pressures used in milling, but Leatherock, McGhee, and Giertz (1937) mention the probable effect of the severity of pressure in grinding.

The temperature plays an important part. With cold rolls Geddes and West (1930) always obtained higher yields and they advocate warming up the rolls, as also does Miller (1937). Ostwald and Steinbach (1928) were interested only in granulation as influenced by temperature in milling with a coffee mill. The different cold milling systems (e.g. Loizillon) do not seem to have been studied with regard to their influence on diastatic activity. Strangely enough Levinson (1935) states that roll temperature has no great practical influence on the temperature of the product under the rolls. He reckoned that the product was in contact with the rolls for only 1/350 second.

As far back as 1879 Brown and Heron found that mechanically injured starch granules are more easily attacked by diastase (reported by Karacsonyi and Bailey, 1930). Blish, Sandstedt, and Kneen (1938) showed that sound, unruptured granules (raw starch) are not attacked by beta-amylase at all. Alsberg and Griffing (1925) state that flours with many injured starch granules, which can be stained e.g. by Congo red, have much material, beta-amylase, dissolved out of the granules. Karacsonyi and Bailey (1930) believe that the increase in diastatic activity due to overgrinding, as measured by the Rumsey autolytic method, is not due to any stimulation of the diastase,

<sup>1</sup> The microphotos of roll surfaces published by the authors are not of the surface itself, but of a cut perpendicular to the surface.



but rather to an increase in accessibility of the starch to the diastase. It is significant that in spite of this increase, gas production was not substantially modified by overgrinding. Pascoe *et al.* (1930) found an increase in diastatic activity equal to 35% after regrinding flour in a ball mill for 20 hours. Recently it has been shown by Sandstedt *et al.* (1939) that the available starch fraction, termed amyloextrin, is also very important as a factor influencing the handling quality of a dough.

Pulkki (1938a) thinks that the starch cells of wheat are surrounded by a film or a thin layer of substance which is easily removable by means of mechanical treatment. The explanation of the British Flour Millers' Research Association, however, seems to be better. Kent-Jones (1939) reports on their work according to which starch cells are probably ruptured around the edges, and starches probably show different degrees of resistance to the mechanical attack during milling.<sup>2</sup>

### Apparatus

Figure 1 shows the modified experimental mill used for most of the tests here reported. The rolls could be very quickly changed, which was of special interest in the case of tests done at different temperatures.

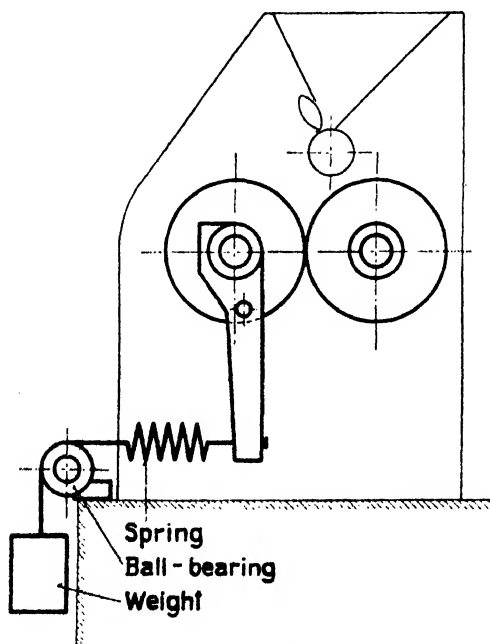


Fig. 1. Laboratory mill for working at a given pressure.

<sup>2</sup> Since the present paper was written C. R. Jones (Cereal Chem. 17: 133-169, 1940) has published results of outstanding importance on the work of the Association referred to. He differentiated between two factors. One of them, the "surface factor," is due to shearing or scraping of material from particle surfaces. This factor is influenced by roll surface and differential roll speed. On the other hand, there is the "internal factor," due to crushing or partial flattening of larger particles. Here the maltose figure depends on the size and hardness of the particles and on the roll pressure.

The main feature is the use of weights instead of springs to apply pressure. The strong spring shown in Figure 1 was included only in case some hard substance might get in between the rolls, but had no effect on the actual pressure. Exact pressures could be applied since, in addition to the weights, each of the two rolls was driven by a separate belt. With the use of gearing at the one end of the rolls—the usual practice in Europe—exact measurement of roll pressure can only be made by making allowance for the one-sided pressure caused by the gearing. For instance, when developing the Buhler roll-pressure applying and indicating device, by which any pressure can be set by a mere movement of the hand and kept constant, special attention had to be paid to this one-sided additional variable. In the mill (Fig. 1) the rolls had a diameter of 6 inches (150 mm.), the usual diameter used in experimental work; the length was 8 inches (200 mm.).

Three widely different roll surfaces were chosen. The term "polished" is used in this paper to designate a pair of new smooth rolls, whereas the rolls which are here termed "frosted" were treated with a sand blast. The degree of roughening depends of course on the hardness of the roll and the microscopical texture of the surface. The "scratched" rolls are those to which cross-cuts of 1 to 3 mm. length had been applied at 45° and 135° to the axe. The Brinell hardness was 555, which is very hard, but it is not implied that this is the optimum. The pressures used were 246, 465, 688, and 908 kg. per meter of roll length. These were actually obtained by adding the following weights to each end of the roll (Fig. 1): 7.7, 14.5, 21.5, and 28.4 kg. In commercial milling of the type of product used in our tests, a pressure of 600 to 700 kg./m. would be normal under European conditions. In some cases, on other products, pressures up to 1300 kg. have already been measured.

The speed of the fast roll was in all cases 340 rpm., which is slow for American conditions. Both in Europe and America 1 : 1.5 is a normal differential for the reduction rolls. In our tests 1 : 1 and 1 : 2.4 were also applied.

For the experiments on roll temperature effect the whole rolls were placed in a hot or a cold water bath for one hour before thorough drying and use in the mill. No arrangement was available for keeping the roll surface temperature constant.

The feed was kept constant, by setting the feed gate. The air in the milling room was 18°C. and had a relative humidity of 55%, except for Test 3. For one test the Buhler automatic laboratory mill was used.

### Material and Methods

Most of these preliminary tests were done with coarse middlings of medium hardness going to the third reduction of a commercial mill, having a moisture content of 14.0%. In some cases flakes were produced, which were eliminated by gentle rubbing. In all cases the product was milled only once, so that the contact with the rolls would only be approximately 1/100 second. The maltose tests were made on the whole product.

With the autolytic saccharification in flour the limiting factor is the quantity of readily available starch present (Blish, Sandstedt, and Kneen, 1938), but—the same authors go on to say—autolytic flour “maltose values” represent approximately a 60% conversion of the available starch fraction.

Besides the Blish and Sandstedt ferricyanide maltose determination, the Berliner and Schmidt (1933) colorimetric maltose method, which is calibrated against the Berliner and Rüter (1928) titrimetric method, was used. Since the autolysis in this last case was only done with a 10% flour-water suspension for 30 minutes at 25°C. much lower values were obtained than by the method of Kent-Jones. The latter advocates a maltose figure between 1.8% and 2.3%, which would correspond to 1.0% and 1.4% by the other method. The colorimetric method is now done with a 20% flour-water suspension, which is digested for 60 minutes at 27°. Fifteen cc. of filtrate is boiled with 5 cc. of normal NaOH. Maltose as well as glucose and levulose are caramelized, but sucrose is not. The depth of yellow-brown color is correlated to the maltose figure. Because of its extreme simplicity this colorimetric method is widely applied in certain parts of Europe. At first a series of standard colored liquids in sealed tubes was used; now the comparison is usually made with a calibrated photoelectric cell (Berliner and Kranz, 1937). Schmidt (1938) reported on further aspects of the method.

The ash determinations were made at 600°C. by the direct ashing method. The temperatures of the stationary rolls were measured with a very fine thermocouple specially constructed to give a good contact with the surface.

### Experimental

*Test 1. Surface, differential, and pressure:* For this test one pound of product was milled under each of the 36 different conditions. Temperature was not considered in this first test, which was carried out six years ago. The results are shown in Figure 2 and Table I. The figure shows that with a very smooth surface even a high differential and a high pressure yields only a low maltose figure, in spite of the

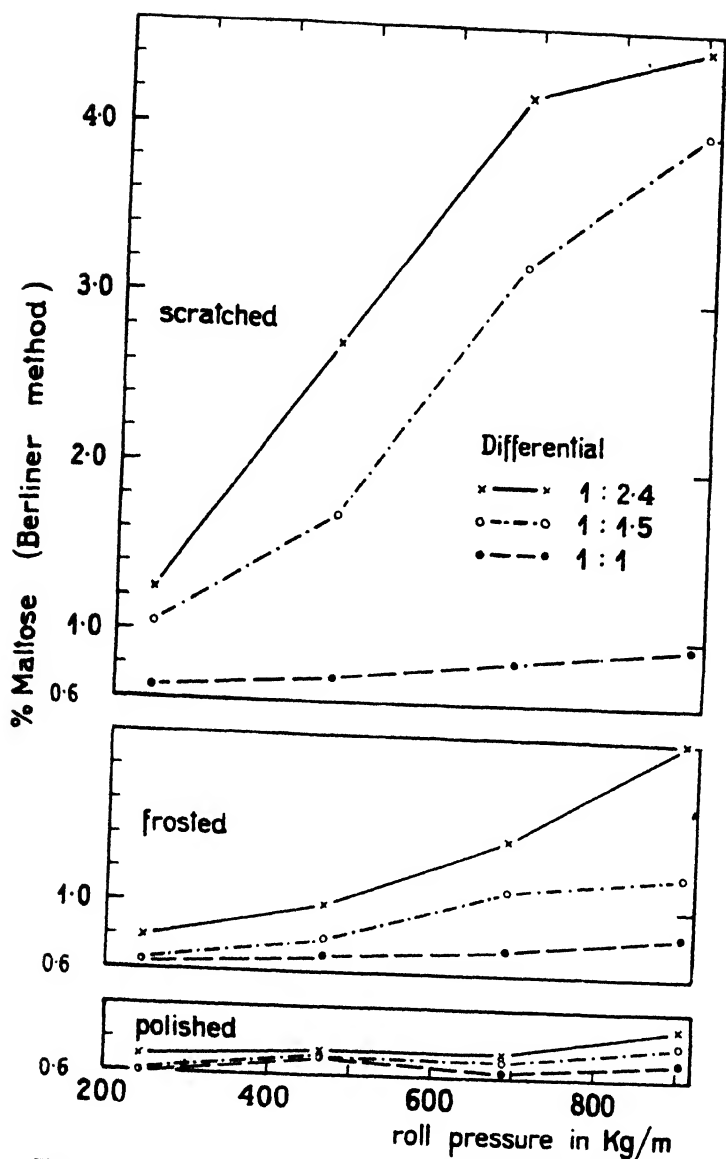


Fig. 2. Influence on maltose figure of surface, pressure, and differential.

TABLE I  
INFLUENCE OF ROLL PRESSURE AND DIFFERENTIAL ON DIASTATIC ACTIVITY  
WITH THE "SCRATCHED" ROLL SURFACE

Data represent mg. maltose per 10 g. flour (Blish and Sandstedt method)

Differential	Roll pressure in kg./m.			
	246	465	688	908
1 : 1	120	—	150	—
1 : 1.5	—	201	244	—
1 : 2.4	198	240	—	312

fact that in this case a fairly high temperature was developed. Naturally it was the scratched surface which produced the highest maltose figures. With this surface, even with a normal differential and pressure, high values are obtained.

The increases shown in Table I and measured by the Blish and Sandstedt method are not so large in percentage as with the Berliner method. Whereas diastatic activity increased constantly, this cannot be attributed to greater fineness alone, because there is a maximum pressure for a product above which no greater fineness can be obtained.

*Test 2. Surface and ash:* This test was done with the Buhler automatic laboratory mill, with which the same sample of Manitoba 2 wheat prepared in exactly the same way was milled. The rolls were set to a definite distance in this case, on the three reduction streams

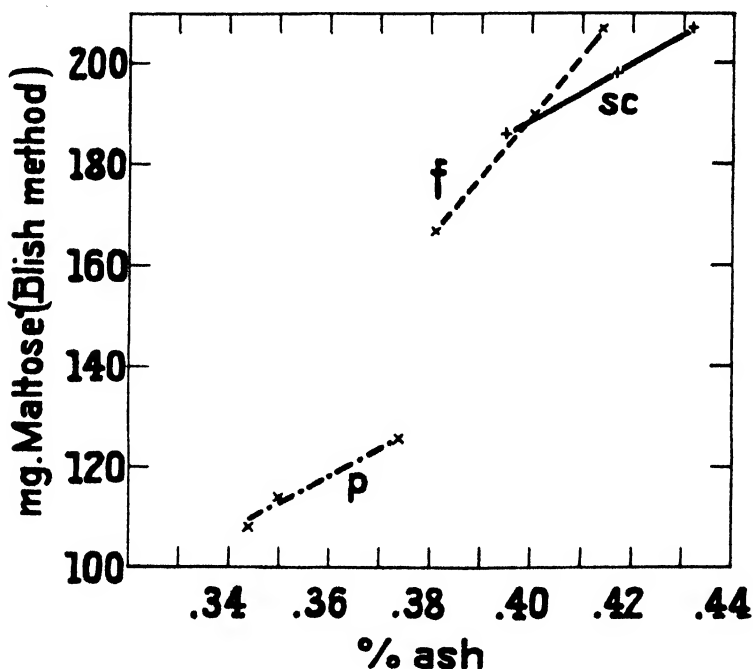


Fig. 3. Influence of roll surface on maltose figure and ash content (13.5% moisture basis). *sc* = scratched surface. *f* = frosted surface. *p* = polished surface. The lowest ash value of each curve is for first-reduction flour alone. The middle value for first- + second-reduction flour. The highest value is for a mixture of all three reduction flours.

to 0.07, 0.05, and 0.03 mm. Figure 3 shows the relationship of maltose to ash for the three different surfaces. The flour yields were:

Surface	Total yield in reduction flour
Scratched	49%
Frosted	46%
Polished	40%

The superiority of the rough surfaces for diastatic activity and yield is clearly seen and cannot be due only to the higher ash content; otherwise the curves marked *sc* and *f* would be in the prolongation of curve *p*.

**Test 3. Roll temperature:** The smoother the roll surface the more heat is developed during milling. After milling for one hour in the Buhler mill at a given setting (rolls not touching) and capacity the following temperatures were measured:

Scratched surface (cool, exact temperature not measured)

Frosted surface 40°

Polished surface 55°

The following test was done with the mill shown in Figure 1, using the frosted surface, 690 kg./m. and a differential of 1 : 1.5. The product used here had a moisture of 15.1% before milling, the room temperature was 15°–17° with an air humidity of 43%, and the barometer was 705 mm. Hg. In each case 2 kg. of middlings were milled. As abscissas of Figures 4 and 5 the average temperatures of both rolls before and after milling were taken. Figure 4 shows that under 45° the rolls had a tendency to warm up, whereas over that temperature a cooling down took place. Flour temperature increased as a linear function of roll temperature between 14° and 41°. This produced a loss in moisture up to 2%, although the product was only milled once.

The increase in maltose—as measured by the Blish method (Fig. 5)—was 42% from 6° to 20° and 32% from 20° to 78°, from 30° onwards the increase being fairly linear.

### Discussion

Table II summarizes the results obtained. The effect of tempera-

TABLE II  
SUMMARY OF THE RESULTS OBTAINED

Maltose figure	Pressure	Differential	Surface	Roll temperature
High	Medium	Medium	Rough	—
"	High	High	Medium	—
"	Medium	Medium	Medium	High
Low	High	None	Rough	—
"	High	High	Smooth	— <sup>1</sup>

<sup>1</sup> Under these conditions roll temperature would be fairly high.

ture alone is not yet quite clear. There may be an activating influence on one of the enzymes, but the greatest effect is no doubt on the starch.

Most authors have given figures showing that the diastatic activity of flour from laboratory-milled wheat is only about 65% of that ob-

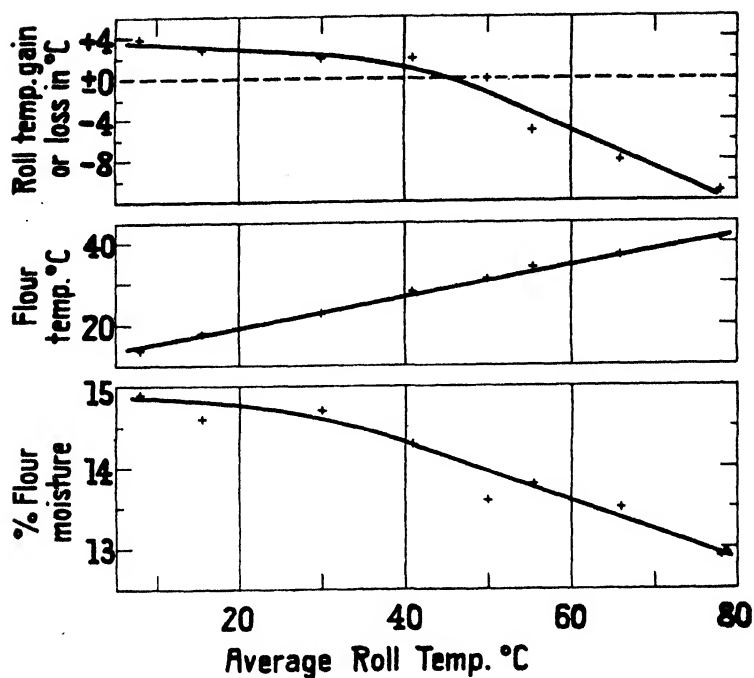


Fig. 4. Roll-temperature difference between beginning and end of milling (above), average roll temperature as a function of flour temperature (middle), and of flour moisture (below).

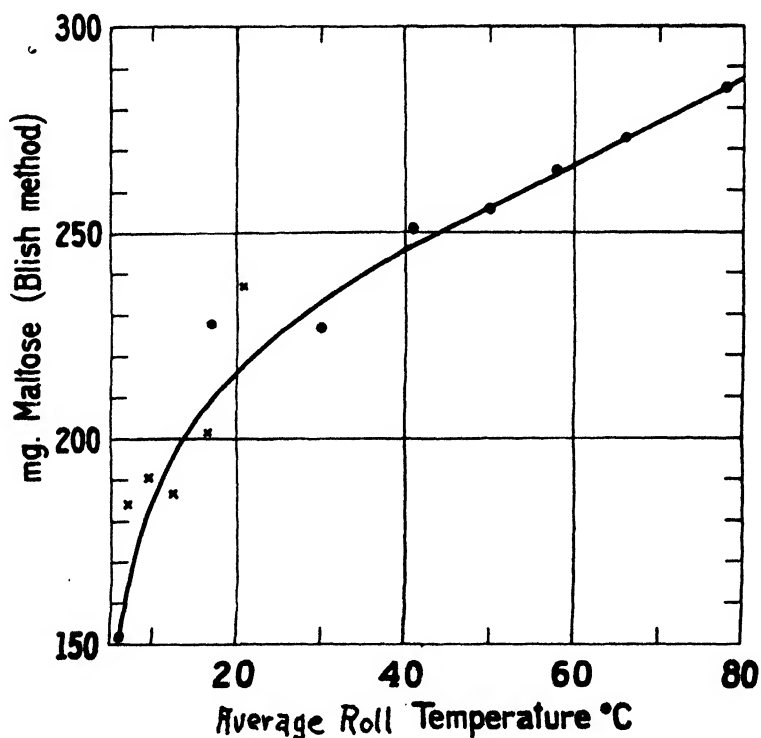


Fig. 5. Influence of average roll temperature on diastatic activity. Crosses imply duplicate and dots quadruple sugar determinations. "Frosted" surface.

tained by commercial milling. The curves of Anderson (1938), who compared commercial flour to flour from the Buhler laboratory mill, show that in some cases there is good agreement in diastatic activity and gas production; in others again the usual lower values for the laboratory flour occur. In view of the results discussed here and of the fact that in some American commercial mills reduction rolls are allowed to run very warm, it is believed that temperature differences at least explain part of the discrepancy found between commercially and laboratory milled flour. It may be that in the case of large mills which maintain cool reduction rolls the correlation of the maltose figure with that of experimentally milled flour is better than average.

To the effect of temperature must be added that of surface structure of rolls. The present study confirms the statement by the author (1938) that laboratory milled flours can be obtained with a normal diastatic activity and that it is a question of choice of roll surface. However other factors, such as gluten quality, do not permit the use of the roughest possible surface.

### Summary

The main factors involved in milling have been enumerated. A laboratory mill has been described, which permits the accurate measurement of roll pressure. The normal pressure used in the milling of coarse middlings is about 600–700 kg. per meter roll length.

Middlings were milled using three different roll surfaces, roll pressures between 250 and 900 kg./m., three different differentials and roll temperatures between 6° and 78°C.

In the absence of differential or with a polished surface no significant increase in diastatic activity was caused by milling, whatever the pressure and surface in the first case, or the pressure and differential in the second case.

In the presence of a differential, the rougher the surface and the higher the roll pressure the greater the increase in diastatic activity.

The smoother the reduction rolls, even if they do not touch each other, the more heat they develop in milling. Roll temperature below 20°C. had more effect on diastatic activity per degree increase than above that temperature. From about 30°C. to 80°C. the increase in maltose figure seemed to be a linear function. Although the product was only in contact with the rolls for approximately 1/100 second the effect of milling on diastatic activity was very noticeable.

The higher maltose figures of commercially milled flours, as compared with experimentally milled flours, doubtless result in part at least from the higher roll temperatures involved in the former process.



### Acknowledgment

The author was assisted in the experimental part by F. Brassel and with the mechanical part by E. Oetiker and wishes to acknowledge their help.

### Literature Cited

- Alsberg, C. L., and Griffing, E. P.  
1925 Effect of fine grinding upon flour. *Cereal Chem.* 2: 325-344.
- Anderson, J. E.  
1938 The Buhler mill. *Northwestern Miller*, Prod. No., Oct. 12, pp. 34-36.
- Berliner, E., and Kranz, W.  
1937 Kolorimetrische Messungen als Schnell- und Mikrobestimmungen. *Mühlenlab.* 7: 89-94.
- and Rüter, R.  
1928 Titrimetrische und polarimetrische Zuckerbestimmung. *Z. ges. Mühlenw.* 5: 116-120.
- and Schmidt, E.  
1933 Über Biuretreaktion, Trommersche Zuckerprobe und eine neue, einfache kolorimetrische Zuckerbestimmung. *Mühlenlab.* 3: 187-192.
- Blish, M. J., Sandstedt, R. M., and Kneen, E.  
1938 The cereal amylases with reference to flour and malt behavior. *Cereal Chem.* 15: 629-657.
- Dedrick, B. W.  
1924 Practical milling. National Miller, Chicago.
- Geddes, W. F., and Frisell, B.  
1935 An experimental flour mill for 100-gram wheat samples. *Cereal Chem.* 12: 691-695.
- and West, H. E.  
1930 A study of the reliability of the experimental milling test. *Sci. Agr.* 10: 333-343.
- Gründer, W.  
1935 Der Einfluss der Korngröße auf Kleber- und Triebeigenschaften bei Weizenmehlen. *Mühlenlab.* 5: 17-26.
- Hopf, L.  
1938 "Taschenbuch für Müllerei und Mühlenbau (2nd ed.). Verlag "die Mühle," Leipzig.
- Karacsonyi, L. P., and Bailey, C. H.  
1930 Relation of the overgrinding of flour to dough fermentation. *Cereal Chem.* 7: 571-587.
- Kent-Jones, D. W.  
1939 Modern cereal chemistry (3rd ed.). Northern Publishing Co., Ltd., Liverpool.
- Kettenbach, F.  
1922 Müllerei und Mühlenbau. Verlag Degener, Leipzig.
- Kosmin, P. A.  
1921 Flour milling. Routledge and Son, London.
- Leatherock, L. E., McGhee, H. W., and Giertz, J. W.  
1937 Diastatic activity in connection with flour mill control. *Cereal Chem.* 14: 161-181.
- Levinson, —  
1935 Etude sur la température des produits qui sortent des appareils à cylindres. *Bull. école française de meunerie*, No. 44 and 45.
- Miller E. S.  
1923 Observations on milling. National Miller, Chicago.  
1937 The purpose of experimental milling. *Bull. Assoc. Operative millers* p. 838.
- Ostwald, W., and Steinbach, W.  
1928 Beiträge zur Kenntnis der Mahlgänge. *Z. ges. Getr.* 15: 72-77. Also *Koll. Zeit.* 43: No. 3.
- Pascoe, T. A., Gortner, R. A., and Sherwood, R. C.  
1930 Some comparisons between commercially and experimentally milled flours. *Cereal Chem.* 7: 195-221.
- Pulkki, L. H.  
1938 Über die Einwirkung des Ausmahlungsgrades und der Vermahlungs-methodik auf die Mehlbeschaffenheit. *Mühlenlab.* 8: 49-52.

- 1938a Particle size in relation to flour characteristics and starch cells of wheat. *Cereal Chem.* **15**: 749-765.
- Raym, W., and Scharffenberg, E.  
1932 Härte und Griffigkeit bei Hartgusswalzen und sonstigen Mahlkörpern. *Z. ges. Getr. und Mühlenw.* **19**: 81-84.
- Sandstedt, R. M., Jolitz, C. E., and Blish, M. J.  
1939 Starch in relation to some baking properties of flour. *Cereal Chem.* **16**: 780-792.
- Schmidt, E.  
1938 Eine kolorimetrische Zuckerbestimmung. *Mühlenlab.* **8**: 121-132.
- Willard, J. T., and Swanson, C. O.  
1911 Milling tests of wheat and baking tests of flour. *Kansas State College Bul.* **177**.
- Ziegler, E.  
1938 The use of a purifier in experimental milling. *Cereal Chem.* **15**: 663-671.

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## FACTORS WHICH INFLUENCE THE PHYSICAL PROPERTIES OF DOUGH. I. EFFECTS OF AUTOLYSIS ON THE CHARACTERISTICS OF DOUGH MIXER CURVES<sup>1</sup>

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(Read at the Annual Meeting, May 1940)

The variability of curves made by a recording dough mixer from different flours, even from the same variety, has been shown by Swanson (1939). The determination of factors that may cause curves to vary is the object of the present series of studies.

Dough has three principal physical properties: plasticity, elasticity, and viscosity. These properties merge into each other, and, as a result of this merging, each characteristic cannot be isolated to permit independent study. Because of this, the experimental measures of the physical properties of dough do not have the same definiteness as if each one of these main properties could be measured by itself and uninfluenced by the others. The characteristics of a dough-mixer curve are the resultant of the changing plastic, elastic, and viscous properties of the dough while being mixed. During that period of mixing which is represented by the ascent of the curve, the water is brought into contact with and is adsorbed by the starch granules and the protein which forms the gluten. During this period, because of the stretching, folding, and restretching action of the moving pins in the mixer, the gluten strands become oriented into a more or less parallel pattern and this continuously developing arrangement increases the resistance to the movement of the pins through the

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<sup>1</sup> Contribution No. 68, Department of Milling Industry.

dough because of increase in elasticity and viscosity. The spreading of the water films over the starch granules and adsorption on the granules are the chief causes of the development of plastic properties. Starch does not contribute to the elasticity; this inheres in the gluten strands.

The viscous properties are mainly inherent in the water films which cover both the starch granules and the gluten strands. Maximum resistance to the movement of the pins through the dough occurs when there is the greatest parallel arrangement of the gluten strands in the dough and before the strands start to disintegrate. The curve reaches the top at the stage of maximum resistance. The rate of increase in resistance and the duration of maximum resistance, giving sharp, rounded, or flat-top curves, is a variety characteristic. When the resistance decreases, the curves start on the down slope. The steepness of the down slope depends upon the rate of decrease in resistance, and this also is a variety characteristic.

These characteristics (rate of increase in resistance, duration, and rate of decrease) are related to inheritance and can thus be used in the evaluation of varieties. The curves are also influenced by the amount of water used in mixing the dough, by the protein content, and by the adjustments on the mixer. Hence, all these must be carefully considered in the interpretation of the curves.

The cause of the decrease in resistance is probably the disintegration of the gluten strands. Hale (1939) presents evidence that "proteinas" is present in wheat flour and that it is an enzyme of the papain type. Markley and Bailey (1938) cite statements which indicate that wheat germ, glutathione, water extracts of yeast, wheat malt, and proteolytic enzymes increase the mobility, that is, decrease the resistance. Cysteine accelerates the rate of decrease, while bromate retards it. Overmixing the dough past the point of maximum resistance gives a measure of mechanical damage to the gluten structure. They also indicate that the effect of wheat malt in increasing the mobility is due almost entirely to alpha-amylase rather than proteases and that it is possible that in some flours the only force active in increasing the mobility upon prolonged mixing is that inherent in the starch. Markley (1937) has shown the thixotropic nature of the starch-water systems. Skovholt and Bailey (1935) found that nonprecipitable nitrogen increased with the extent of mixing and if proteases were added this was much larger. However, the increase in precipitable nitrogen was too small to be commensurate with the physical breakdown of the dough. The changes in physical properties are thus shown to be due to both chemical and physical forces.

Cairns and Bailey (1928) digested a mixture of 25 g. of flour and 100 cc. of toluene water. After five hours of digestion of first middlings flour + 0.1 g. of trypsin, the decrease in viscosity was from 125, control, to 12 W&T°. Without added protease the change after 24 hours of digestion of straight winter wheat flour was from 145, control, to 40 W&T°. When 3% of sprouted wheat flour was present the decrease was from 121 to 35 W&T°. Thus there are substances in the flour itself which decrease the viscosity, but the rate is much less than when proteases are present.

Some random curves made in this laboratory showed that flour-water doughs undergo changes after mixing and incubation, and that these changes are reflected in the characteristics of the curves made on the incubated doughs. Further, the extent of these changes becomes greater with the duration of the time of incubation. The changes found are characterized by the resistance to mixing, rapidly reaching a maximum and a greater rate of decrease in resistance. These changes might be caused by the action of proteases on gluten, by liberating phosphatides which would decrease viscosity of the water films that form a continuous system through the dough, by the increase in the amount of free water in this system. Scott Blair (1938) states: "Flour suspensions lose viscosity on standing and so do doughs. . . . it seems that the protein takes up more water than it can afterwards hold. As it gradually gives up this water, the starch gains water, but does not immediately immobilize it in the same way as does the proteins and the viscosity falls. It is for this reason that doughs soften during fermentation."

There are, thus, several ideas as to the causes of the changes which occur in the flour-water doughs upon standing and which are shown in the characteristics of curves made on such doughs. One theory is that the changes are due to enzymes and the other is that they are due to a type of syneresis which would increase the freedom of flow of dough substances on each other. If the changes are due mostly to enzymes, then they should be influenced by inhibitors.

There is also the possibility that the decrease in resistance is due to mechanical tearing of the gluten strands as soon as the remixing starts on the incubated doughs. It is also probable that several of these causes are operative at the same time, making the solution of the problem more difficult.

### Experimental Procedure

The general procedure in these experiments was to mix the flour-water doughs until the water was incorporated, remove the dough from the mixer, place it in a small dish, cover and place in a cabinet

controlled at 30°C. This treatment in the cabinet subjects the dough to what may be called autolysis.<sup>2</sup> After predetermined periods of incubation, the dough was replaced in the bowl of the National Swanson-Working recording mixer<sup>3</sup> and the curves obtained. This mixer requires only 35 g. of flour for each curve. Optimum absorption and a constant machine setting were used. The periods used at first for incubation were  $\frac{1}{4}$ ,  $\frac{1}{2}$ , 1, 2, 3, and 4 hours, but it was soon found that periods longer than two hours served no useful purpose. Several modifications or additions to this procedure were used which will be explained in connection with the several experiments. The absorption was so controlled that the consistency or "feel" of the dough was very similar to that of doughs used in baking. Not all curves obtained are presented in the figures which are given, but a selection was made of those curves which best illustrated the changes in the dough in connection with a particular experiment. Control curves were obtained for each important group.

The flours selected for this study were from Tenmarq, Turkey, and Chiefkan wheats and were chosen because it was known that their doughs had very pronounced differences in physical properties and hence would give curves of varying characteristics. The moisture, protein, and ash (not corrected for moisture) on these flours were as follows:

	Moisture, %	Protein, %	Ash, %
Tenmarq	12.62	12.81	.400
Turkey	13.14	13.05	.448
Chiefkan	13.61	12.80	.442

### Effects of Autolysis on Curves

The control curves and the curves obtained after  $\frac{1}{2}$ , 1, and 2 hours of incubation are shown in Figure 1. Curves were also obtained after 3 and 4 hours of autolysis and were very similar to those obtained after 2 hours. Considerable changes had taken place in the  $\frac{1}{4}$ -hour period and the  $\frac{1}{4}$ -hour curves differed from the  $\frac{1}{2}$ -hour curves only in showing less amount of change. It is evident that the greatest amount of the change took place in the first half hour and that among the varieties, Chiefkan shows the greatest amount of change, Tenmarq the least, and Turkey intermediate. The three curves in the bottom row were included here to show the effect on Turkey flour of 12 mg. of papain added to the water at the time of the first mixing. These

<sup>2</sup> Autolysis is used here to designate the process resulting in the automatic disintegration of substances such as gluten or starch similarly to what happens when the presence of enzymes is known.

<sup>3</sup> The movement of the pen and chart paper in this mixer is such that the curves resemble those made by the Swanson-Working larger machine (Swanson, 1939). For a description of this machine see *Cereal Chemistry* 10: 11-21.

curves were made after the doughs had been incubated for 5, 10, and 15 minutes, respectively.

The curves from the autolyzed doughs differ from the control in these respects: The pen swung almost to the top of the chart at once, and this was caused by the plasticity yield value when the pins started moving through the dough. The height of this swing cannot be used

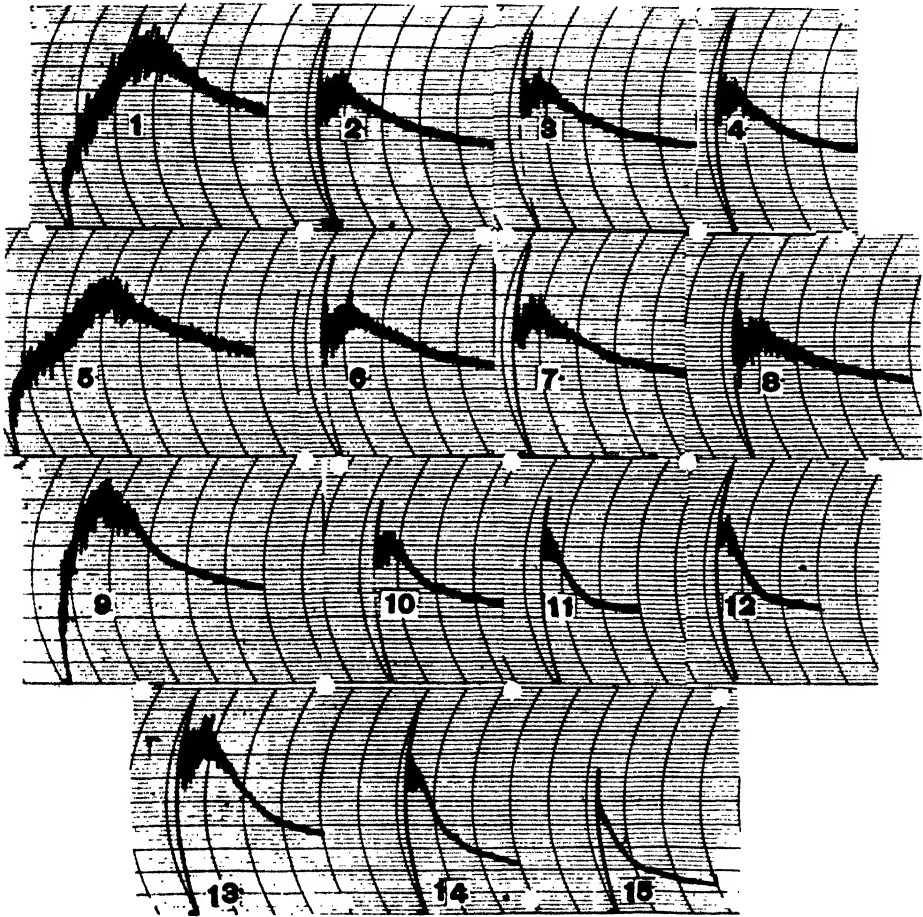


Fig. 1. Effect of autolysis.

(Figures in parentheses refer to the curve numbers.)

Top row, Turkey	(1) Control	(2) $\frac{1}{2}$ hr.	(3) 1 hr.	(4) 2 hrs.
Second row, Tenmarq	(5) Control	(6) $\frac{1}{2}$ hr.	(7) 1 hr.	(8) 2 hrs.
Third row, Chiefkan	(9) Control	(10) $\frac{1}{2}$ hr.	(11) 1 hr.	(12) 2 hrs.
Bottom row, Turkey + papain	(13) 5 min.	(14) 10 min.	(15) 15 min.	

as a comparative measure of yield value because of the inevitable variation in handling when the autolyzed doughs are replaced in the bowl. After the first upward swing the width of the curve assumes the maximum and for Turkey and Tenmarq there is a short upturn, but not for Chiefkan. The rate of decrease in resistance is greater

for the autolyzed doughs than for the controls, especially for Chiefkan. The curves showing the effect of papain are similar to the others with the exception that the change was much greater. The papain produced in five minutes as great an effect on Turkey as autolysis for one-half hour, and in ten minutes the effect was as great on Turkey as autolysis for one hour on Chiefkan.

### **Effects of Varying Protein Content on Autolysis**

The three flours used for the curves in Figure 1 had nearly the same protein content. Would the effects of autolysis be similar on high and low protein flours?

From wheat-variety testing experiments, there were on hand flours from several varieties which varied in protein content. Five flours from Turkey varied in protein from 9.5% to 15.6%; four from Michigan Wonder varied from 10.3% to 14.0%; five from Chiefkan varied from 10.6% to 15.4%; and five from Clarkan varied from 9.9% to 15.4%. Curves were obtained on all these flours both before and after one hour's incubation. From these curves there are shown in Figure 2 those from the highest and lowest protein samples in Turkey and Michigan Wonder and also the curves from the lowest, medium, and highest protein samples from Chiefkan and Clarkan. The check curves are placed in the first and third rows, and the curves from the autolyzed doughs in the second and fourth rows.

The effects of the protein content on the curve characteristics are striking. The tops of the curves from the high-protein flours are much higher than those from the low-protein flours. The protein content does not influence the time required for the curve to reach the top, and this makes the angle of the ascending and descending slopes much greater in the curves from the high-protein flours. The effects of autolysis are relatively the same on the low as on the high-protein flours. Chiefkan and Clarkan show a much greater increase in mobility than Turkey or Michigan Wonder. The conclusion from the curves shown in Figure 2 is that although the protein content has a great influence on the curve characteristics, it is not in itself a factor in the autolysis of the flour and water doughs.

### **Effects of $\text{KBrO}_3$ on Autolysis**

The first thought might be that the changes which take place during incubation are caused by proteolytic enzymes. Their activity would weaken the gluten strands and when the dough is remixed the resistance would be greatly decreased as soon as the plastic yield was overcome. In overcoming the plastic yield, there is a tearing action of the weakened gluten strands. That proteases cause a decrease in

viscosity was shown by Cairns and Bailey (1928). If the cause of the large decreased resistance is due to proteases, then their action should be stopped or minimized by protease inhibitors such as  $\text{KBrO}_3$ . Flohil (1936) reports that  $\text{KBrO}_3$  neutralized the effects of papain and malted wheat flour extract. In the wheat-meal-time-fermentation test it was found that  $\text{KBrO}_3$  greatly increased the time from the

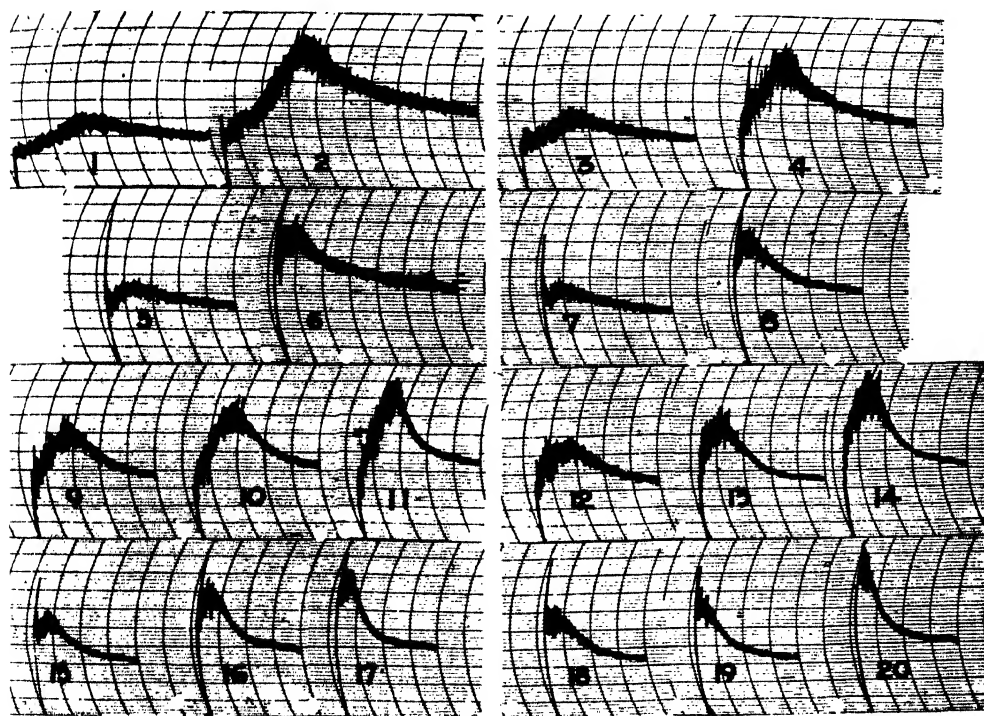


Fig. 2. Autolysis of flours of varying protein content.

	Turkey			Michigan Wonder		
Protein, %	9.5	15.6		10.3	14.0	
Checks	(1)	(2)		(3)	(4)	
Incubated	(5)	(6)		(7)	(8)	
	Chiefkan			Clarkan		
Protein, %	10.6	12.7	15.4	9.9	12.2	15.4
Checks	(9)	(10)	(11)	(12)	(13)	(14)
Incubated	(15)	(16)	(17)	(18)	(19)	(20)

moment the doughball was put on the water until a break was observed at the water-dough interface. It was found that proteases would also markedly decrease this time (Swanson and Dines, 1939).

The effects of using  $\text{KBrO}_3$  are shown in Figure 3. The upper row of curves is from Turkey, the middle row, from Tenmarq, and the bottom row from Chiefkan. Each pair to the left was made using 16 mg. of  $\text{KBrO}_3$  and each pair to the right with 100 mg. The first curve in each pair is the control; the second in each pair is the curve



obtained after one hour of autolysis. Longer and shorter periods were also used for autolysis, but the curves obtained after one hour illustrate what took place.

The presence of 16 mg. of  $\text{KBrO}_3$  slightly increased the duration of the upturn in the curves obtained after autolysis, and with 100 mg. the upturn was even greater, especially for Turkey and Tenmarq. Part of the rise in the curves after autolysis may have been due to the salt effects, especially when 100 mg. were used. As is well known, certain salts, notably  $\text{NaCl}$ , have a stiffening effect on dough. That  $\text{KBrO}_3$  has a marked effect on the dough during mixing has been shown by Baker and Mize (1937). Freilich and Frey (1939) found

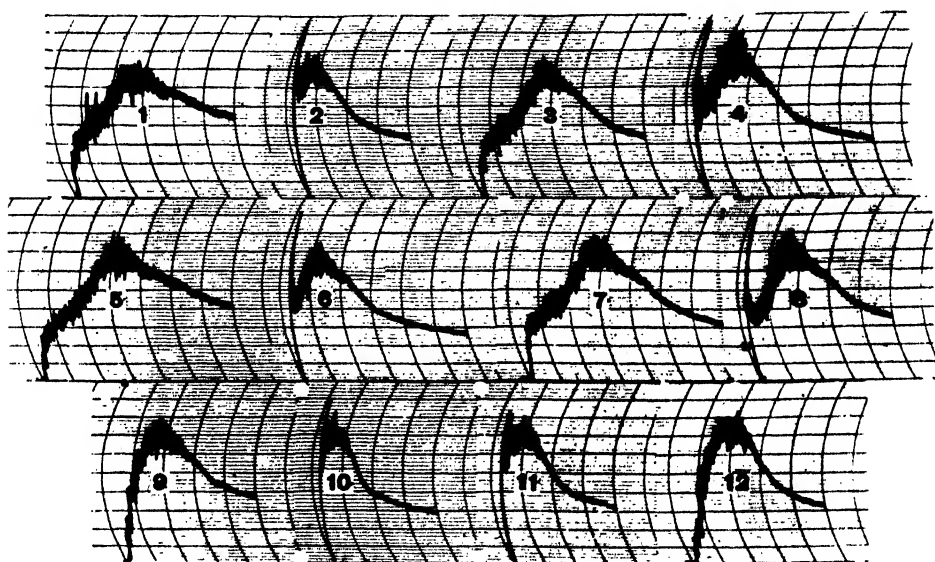


Fig. 3. Effects of  $\text{KBrO}_3$ .

	16 mg. $\text{KBrO}_3$		100 mg. $\text{KBrO}_3$	
Turkey	(1) Control	(2) 1 hr.	(3) Control	(4) 1 hr.
Tenmarq	(5) Control	(6) 1 hr.	(7) Control	(6) 1 hr.
Chiefkan	(9) Control	(10) 1 hr.	(11) 1 hr. <sup>1</sup>	(12) Control <sup>1</sup>

<sup>1</sup> These two have been reversed in position by mistake.

that increasing amounts of  $\text{KBrO}_3$  gradually neutralized the effects of papain. Too large amounts, however, reduced the loaf volume. It thus becomes difficult to say whether there was any inhibiting effect from the  $\text{KBrO}_3$ .

### Effects of $\text{NaCl}$ and $\text{KBr}$

To learn how much the salt effects of  $\text{KBrO}_3$  may have influenced the characteristics of the curves in Figure 3,  $\text{NaCl}$  and  $\text{KBr}$  were added to the water used in mixing the dough. The stiffening effect

of sodium chloride and similar salts on dough is probably due to the adsorbed ions holding around themselves shells of water molecules. This would decrease the amount of water in the free condition and thus diminish the ease of movement which is made possible by the water films on the gluten strands and on the starch granules and hence cause an increase in resistance. Such an effect would also be produced by  $\text{KBrO}_3$ , and this may partly account for the behavior of the curves from the doughs autolyzed with the 100 mg. of  $\text{KBrO}_3$ . This salt, however, has an additional action because of its oxidizing effects which the bromide,  $\text{KBr}$ , would not have. The curves obtained from using  $\text{NaCl}$  and  $\text{KBr}$  are shown in Figure 4. The left three

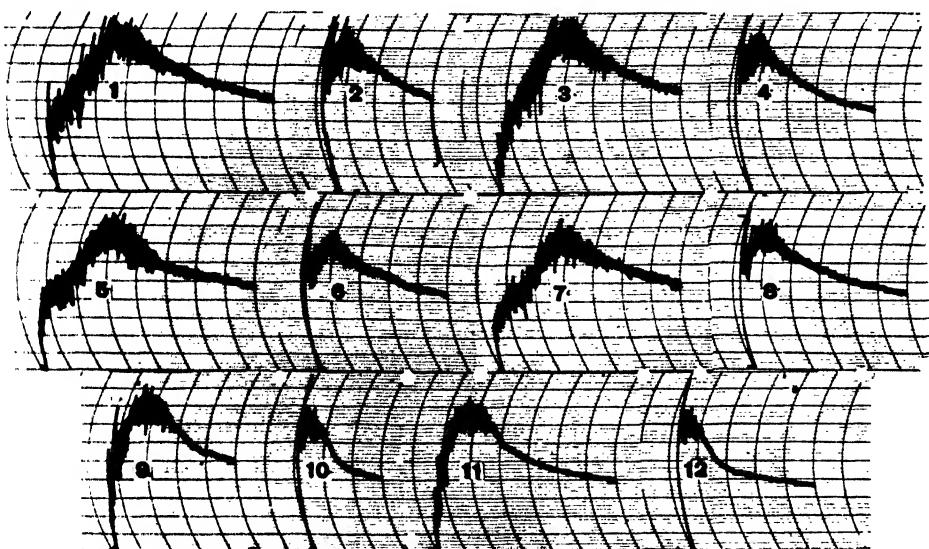


Fig. 4. Effects of  $\text{NaCl}$  and  $\text{KBr}$ .

	100 mg. $\text{NaCl}$		100 mg. $\text{KBr}$	
Turkey	(1) Control	(2) Autolyzed	(3) Control	(4) Autolyzed
Tenmarq	(5) Control	(6) Autolyzed	(7) Control	(8) Autolyzed
Chiefkan	(9) Control	(10) Autolyzed	(11) Control	(12) Autolyzed

pairs show the effect of  $\text{NaCl}$  and the right three pairs, the effect of  $\text{KBr}$ ; the right one in each pair is the curve obtained after one hour of autolysis, and the left is the control containing the salt but obtained before autolysis.

The effects of each of the two salts are very similar, both before and after autolysis. Both the salts produce a stiffening effect shown by the increase in the heights of the curves. These two salts are not protease inhibitors; hence the rise in the curves after autolysis is due primarily to the effects of the salts restricting the mobility by the appropriation of water and hence decreasing the amount in the water

films. The similarities in the curves of Figures 3 and 4 indicate that the  $\text{KBrO}_3$  did not have any protease-inhibiting effect.

Besides proteolysis there are other suggestions as to the cause of increased mobility in the autolyzed doughs. One is that it is perhaps due to a type of syneresis by which water is released from the protein, thus increasing the amount of free water. If the increased mobility is due to this cause, then the addition of starch, which would serve to appropriate this free water, should decrease the mobility. A few such trials were made, but with inconclusive results. Trials were also made to see if squeezing the autolyzed doughs between iron rollers would restore the viscosity, but the results of this were also inconclusive.

### Summary Discussion

Doughs were mixed until the water was incorporated with the flour and then left in a cabinet controlled at  $30^\circ\text{C}$ . for various periods of time. They were then placed in the bowl of the recording dough mixer and the curves obtained. These curves from the autolyzed dough differed very much from the curves obtained immediately after the water came in contact with the flour. The plastic properties were more evident at first. The yield value was such that the pen swung nearly to the top of the chart at once, then swung back and forth, and there was only a small rise in the curve. This rise decreased in relation to the duration of the autolysis. The maximum resistance lasted only a short while when it rapidly assumed the minimum with a steep down slant of the curve. Considerable change had taken place in one-fourth hour, much more in one-half hour, and after two hours there was little further change. These changing characteristics were more evident in Chiefkan than in Turkey, and more so in Turkey than in Tenmarq.

The curves from autolyzed doughs were similar to those obtained with the use of papain. If this change upon autolysis was due to enzyme action, it was only slightly influenced by the presence of  $\text{KBrO}_3$ . There was some "salt effect" from  $\text{KBrO}_3$ , which was shown to be similar to the effects of  $\text{KBr}$  and  $\text{NaCl}$ .

The gradual softening of the dough becomes a factor when doughs are mixed for a long time. The continuous mixing would not have the same effect as remixing after the doughs have stood for some time as was the case with these autolyzed doughs. But since the effects of this standing was marked after 15 minutes and much more so after 30 minutes, it is easily possible that this change starts soon after the dough has reached maximum resistance in the mixing. This softening also becomes a factor when tests are made on doughs which are allowed to stand considerable periods before the tests are applied.

## Literature Cited

- Cairns, A. and Bailey, C. H.  
1928 A study of the proteoclastic activity of flour. *Cereal Chem.* 5: 79-104.
- Baker, J. C., and Mize, M. D.  
1937 Mixing doughs in vacuum and in the presence of various gases. *Cereal Chem.* 14: 720-734.
- Flohil, J. T.  
1936 The effect of chemical flour improvers on proteolytic action in relation to the gas-retaining capacity of fermenting doughs. *Cereal Chem.* 13: 675-689.
- Freilich, J., and Frey, C. N.  
1939 Dough oxidation and mixing studies. I. The action of potassium bromate in dough. *Cereal Chem.* 16: 485-494.
- Hale, W. S.  
1939 The proteinase in wheat flour. *Cereal Chem.* 16: 695-702.
- Markley, Max C.  
1937 The colloidal behavior of flour doughs. I. The thixotropic nature of starch-water systems. *Cereal Chem.* 14: 434-436.
- and Bailey, C. H.  
1938 The colloidal behavior of flour doughs. IV. The causes of the increase in mobility of the doughs upon prolonged mixing. *Cereal Chem.* 15: 708-711.
- Scott Blair, G. W.  
1938 An introduction to industrial rheology (p. 69). P. Blakiston's Son & Co., Philadelphia.
- Skovholt, O., and Bailey, C. H.  
1935 The effect of mixing and fermentation upon the protein structure and the colloidal properties of doughs. *Cereal Chem.* 12: 307-320.
- Swanson, C. O.  
1939 Variations in dough-development curves. *Cereal Chem.* 16: 625-643.
- and Dines, F. T.  
1939 The wheat-meal-time-fermentation test. II. Effect of proteases, protease activators, and protease inhibitors. *Cereal Chem.* 16: 168-177.

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## FACTORS WHICH INFLUENCE THE PHYSICAL PROPERTIES OF DOUGH. II. EFFECTS OF ENZYMES ON CURVE CHARACTERISTICS<sup>1</sup>

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(Read at the Annual meeting, May 1940)

In a previous paper (Swanson, 1940b) it was shown that autolysis or merely incubating dough at 30°C. for various periods after it is mixed has a marked influence on the curve characteristics of this dough when it is remixed. The change was clearly noticeable after 15 minutes, and after one hour or more it was very marked. The curves obtained by remixing the dough after incubating at 30°C. for various periods resembled the curves obtained when papain had been added when the dough was first mixed and the curves run after only a few minutes' rest.

The main difference in procedure was in the length of the incubation

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<sup>1</sup> Contribution No. 69, Department of Milling Industry.

period. While the end results were similar, the effects from papain were produced in a very much shorter time than when the water and flour doughs were autolyzed or incubated. The presence of  $\text{KBrO}_3$  had little effect on the characteristics of the curves made on the autolyzed doughs, other than what may be termed salt effects. That this was a salt effect was shown more clearly by using  $\text{KBr}$  and  $\text{NaCl}$  for comparison. That the effects from autolysis were apparently not due to syneresis was indicated by the negative effects of adding starch or flour which would adsorb at least a part of the freed water in the autolyzed dough. Reworking and pressing the autolyzed dough through iron rolls also had a negative effect, indicating that the broken gluten strands could not be reunited by mechanical means.

In this paper the influences of the presence of enzymes on the curve characteristics are presented. Pepsin, trypsin, and papain were used as representatives of the proteases, diastase and taka-diastase as the amylases, and cysteine monohydrochloride as the protease activators. The effects of extracts of commercial wheat germ were used to compare with the action of the proteases.  $\text{KBrO}_3$ , as a representative of protease inhibitors, was also included in the study.

### Procedure

The enzymes or other materials used were dissolved or suspended in the water so as to be thoroughly distributed while the dough was being first mixed. Each dough or curve was made on the National Swanson-Working recording mixer<sup>2</sup> from 35 g. of flour plus the needed water. Some curves were obtained from doughs without any rest period in order to obtain the effects of the enzymes during mixing, and others were made on doughs after resting or incubating for various periods so as to obtain the time effects.

### Effects of the Three Proteases: Pepsin, Trypsin, and Papain

The three proteases were added in varying amounts starting with 2 mg. and increasing up to 20 mg., and the curves completed without any intervening rest period. The curves showed that amounts less than 12 mg. had no pronounced effects and amounts larger than this served no useful purpose since the curves were very similar to those obtained with the 12 mg. The curves obtained with each of the enzymes are shown in the upper row of Figure 1 for Turkey, and in the third row for Chiefkan. The first curve in each row is the check, the second curve shows the effect of pepsin, the third the effect of trypsin, and the fourth the effect of papain. Without incubation it is evident that on Turkey the least effects were produced by pepsin,

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<sup>2</sup> Made by the National Manufacturing Company, Lincoln, Nebraska.

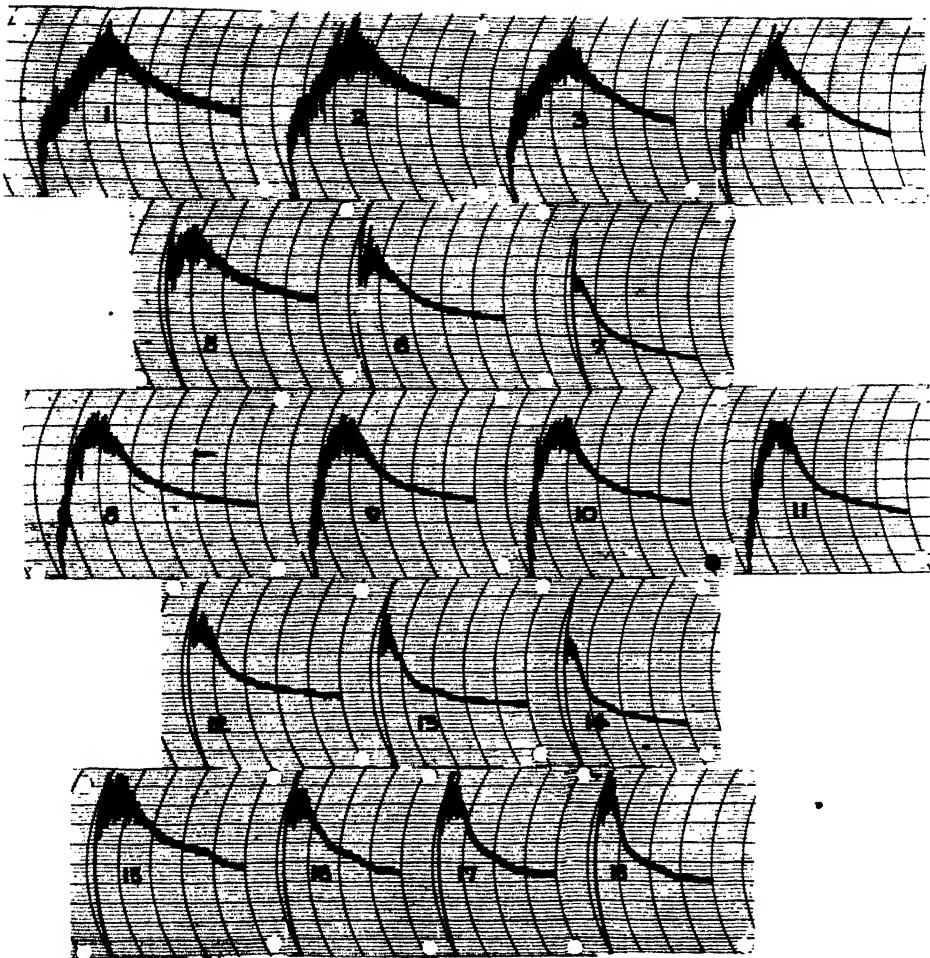


Fig. 1. Effects of the pepsin, trypsin, papain proteases, and extract of wheat germ on curve characteristics.

Turkey	<i>No Incubation Period</i>			
	(1) Check	(2) Pepsin	(3) Trypsin	(4) Papain
	<i>Incubated 10 Minutes</i>			
	(5) Pepsin	(6) Trypsin	(7) Papain	
Chiefkan	<i>No Incubation Period</i>			
	(8) Check	(9) Pepsin	(10) Trypsin	(11) Papain
	<i>Incubated 10 Minutes</i>			
	(12) Pepsin	(13) Trypsin	(14) Papain	
Turkey	<i>Wheat Germ + 10 Min. Incubation Period</i>			
	(15) 5 cc.	(16) 10 cc.	(17) 15 cc.	(18) 20 cc.

the most marked by papain, and that trypsin was intermediate. On Chiefkan both pepsin and trypsin had greater effect than on Turkey, but for papain there was little difference in the effects on the two wheats.

### **Effects of Incubation with the Proteases**

The fact that there was a noticeable effect from the enzyme while the dough was being mixed for the curve, indicated that the time for incubation need not be as long as for autolysis alone. Several trials showed that ten minutes of incubation gave the best results. The curves from the incubated doughs are shown in the second row of curves for Turkey and for Chiefkan in the fourth row in Figure 1. With the incubation it is evident that on Turkey, papain had the greatest effect, pepsin the least, and trypsin had more effect than pepsin. On Chiefkan, pepsin and trypsin had greater effect than on Turkey, while the effect of papain was of the same order. Balls and Hale (1936a) found that the addition of 50 mg. of papain or of glutathione to dough equivalent to a one-pound loaf practically liquefied the dough and the loaf did not rise at all.

### **Effects of the Water Extract of Wheat Germ**

Commercial wheat germ was sifted to remove most of the bran particles. The germ was then ground in a hammer mill to pass a  $\frac{1}{2}$ -mm. sieve. This ground germ was soaked for several hours in water in the proportion of one to five. Thus five cc. of extract represented one gram of the germ. A clear extract was obtained by first filtering through linen cloth and then centrifuging. Portions of this clear but colored extract were used together with enough water to make doughs from the Turkey flours. After the preliminary mixing the doughs were incubated at 30°C. for ten minutes and the curves obtained therefrom are shown in the lowest row on Figure 1. It is evident that the effects on the curves are similar to those obtained with trypsin (6) when digesting for ten minutes. It thus appears that the effects are due to the proteases extracted from the germ or may be due to glutathione which is present in the germ. Sullivan, Howe, and Schmalz (1936) obtained marked modifications in the farinograms both from the water extract of wheat germ and from the extracted glutathione.

### **Effect of Cysteine Monohydrochloride**

Balls and Hale (1936b) measured the rate of softening of flour paste by noting the time required for BB shot to sink below the surface. A paste of whole-wheat flour made with equal parts of flour and water saturated with CO<sub>2</sub> and incubated at 30°C. for 30 minutes presented a surface through which the shot took six minutes to sink. After the same flour had been bleached with chlorine, the time was increased to five hours. The addition of one mg. of cysteine per gram of dry flour reduced the time for the untreated flour to 20

seconds and to 80 seconds for the chlorine bleached flour. The authors state: "It is not a foregone conclusion that the liquefaction of the paste is due to a proteolytic ferment." They found also that kneading 40 g. of wet gluten with 100 mg. cysteine and incubating at 45°C. for 30 minutes liquefied the gluten to a milky white fluid that could be diluted with water. Thus the presence of the cysteine induces the peptization of the gluten and this in turn should increase the mobility of a dough as shown by the recording dough mixer curves.

The trial curves made with cysteine present showed that the rate of action was much more rapid and the amounts needed were much less than with papain. The effects on the curves when various amounts of cysteine were present in the dough are shown in the upper three rows of Figure 2. These curves were obtained respectively from Turkey, Tenmarq, and Chiefkan with 0, 3, 6, and 12 mg. of cysteine present and no intervening incubation period. It is evident that the mobility increases with the amounts of cysteine and that even 3 mg. produce very marked changes during the time that the dough is being mixed. It seems that part of the effect is immediate since the upslope of the curve is notably steeper than in the control curves.

Since 3 mg. of cysteine produced marked effects during the mixing, this amount was used with short incubation periods. It was found that a 10-minute period such as was used with the proteases reduced the dough to a sticky paste, too fluid to handle in the mixing bowl. Several trials showed that the mixing period and the rest period combined should not be more than 2 minutes. Turkey was mixed  $\frac{3}{4}$  minute, Tenmarq 1 minute, the Chiefkan  $\frac{1}{2}$  minute, and the rest periods were such as to make the total 2 minutes. The curves obtained on the three flours are given in the fourth row of Figure 2. The effects were almost the same on each of the three flours and were like that obtained with 12 mg. of papain after 10 minutes incubation (Curves 7 and 14, Figure 1).

Three curves from Turkey, Tenmarq, and Chiefkan are presented in the last row of Figure 2 to compare the effects of incubating the plain flour and water dough at 30°C. for several hours with the effects of cysteine acting for two minutes before beginning to run the curve. The Turkey and Chiefkan curves show about as great an effect from autolysis alone, as when cysteine was present, and the Tenmarq curve almost as much effect, but the effects from autolysis took several hours while only two minutes were required to produce the effects when cysteine was present.

Thus the same effects on the curves were produced by proteases, by cysteine, by autolysis, or by simply incubating the water and flour doughs for several hours. The main difference was in the time



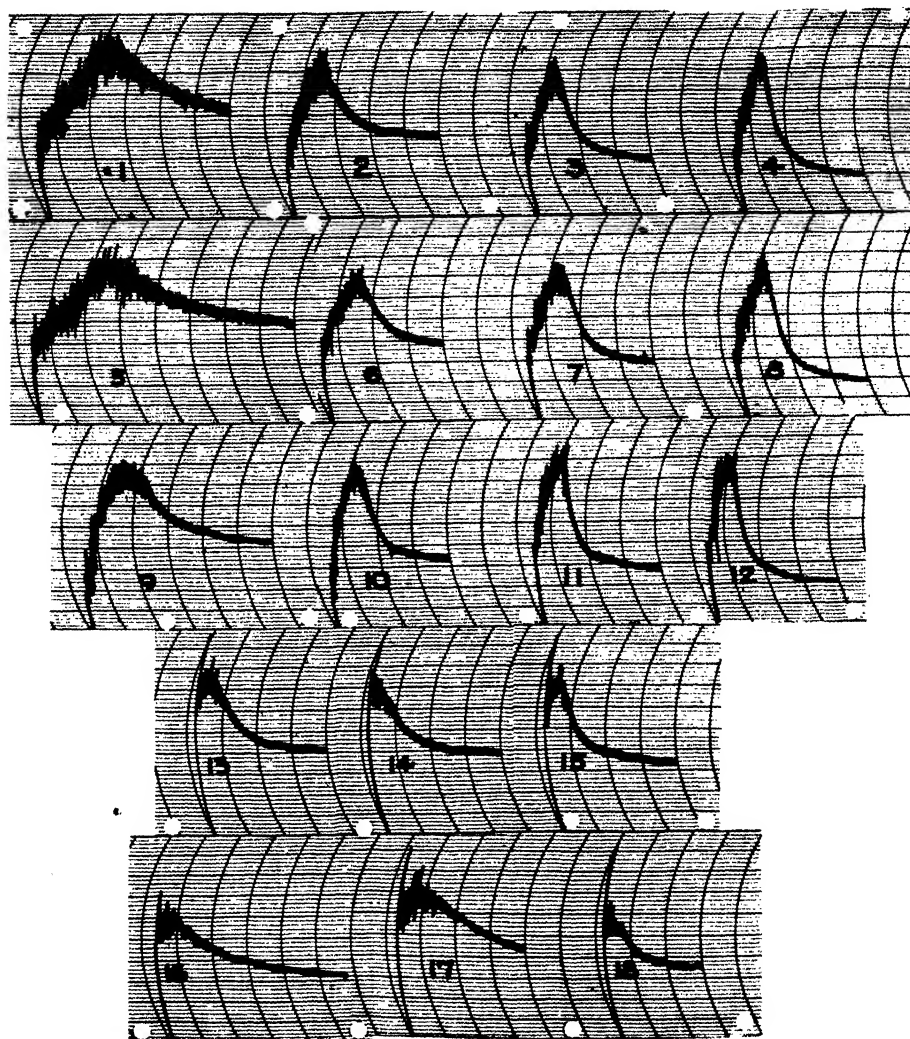


Fig. 2. Effects of cysteine.

*No Incubation or Rest Period*

## Milligrams cysteine

	0	3	6	12
Turkey	(1)	(2)	(3)	(4)
Tenmarq	(5)	(6)	(7)	(8)
Chiefkan	(9)	(10)	(11)	(12)

*Short Rest Period (3 mg. cysteine)*

(13) Turkey (14) Tenmarq (15) Chiefkan

*Several Hours' Autolysis*

(16) Turkey (17) Tenmarq (18) Chiefkan

required. Cysteine produces the effect in the shortest time and with the least quantity. Papain requires five times as long as cysteine and autolysis alone requires from six to twelve times as long as papain. Balls and Hale (1936b) present the statement that the effect of cysteine

on gluten is not the activation of an enzyme but an action on a protein. The extreme speed of the reaction would favor this view. On the other hand, Jørgensen (1936) presented evidence to show that wheat flour contains powerful but inactive proteolytic enzymes. "Usually these enzymes are latent, but an activator such as glutathione which in itself does not possess proteolytic activity is able to stimulate the proteinases of wheat flour." Read and Haas (1937a) state that excessive dosages of a "proteinase" or some "proteinase" activator such as glutathione or cysteine hydrochloride will ruin the baking properties of any gluten. "A stiff dough may readily be reduced to the consistency of a thick soup."

Glutathione, according to Hopkins (1929), is a tripeptide,  $C_{10}H_{17}N_3SO_6$ , containing glycine, glutamic acid, and cysteine, thus indicating the possibilities of similarities in reaction between glutathione and the cysteine monohydrochloride. It is not illogical to suppose that cysteine may activate both the protein and the proteases. This double activation would, in part at least, help to account for the speed of the action of cysteine.

### Effect of $KBrO_3$ on Pepsin and Papain

Potassium bromate is considered a protease inhibitor. The use of  $KBrO_3$  in connection with the wheat-meal-time-fermentation test greatly increased the time from the moment the doughball was put into the water until disintegration could be observed at the dough-water interface (Swanson, 1940a). Flohil (1936) reports that 15 mg. of  $KBrO_3$  neutralized the effects of 15 mg. of papain and that 30 mg. inhibited the effect of malted wheat flour extracts. Read and Haas (1937b) present data which fail to support the opinion "that the benefits commonly derived from the use of bromate in flour doughs result directly from its inhibitive action on the 'proteinase' contained in the flour." They found that bromate did not adversely affect the proteolytic power of malt, pepsin, trypsin, or taka-diastase when these products were allowed to act on gelatin. These authors have made further studies (Read and Haas, 1939) on the activation and inhibition of flour "proteinase." They report that  $KBrO_3$  had a marked inhibition on papain but not on trypsin as measured by the liquefaction of gelatin.

The effect of  $KBrO_3$  was obtained by adding 12, 24, 36, and 72 mg. portions in combination with either pepsin or papain each used in 12-mg. amounts, and the curves are given in Figure 3. The first row of curves shows the effect of the various amounts of  $KBrO_3$  with pepsin, using no rest or incubation period. These curves are similar to curve (2) in the first row of Figure 1, where pepsin alone was used.

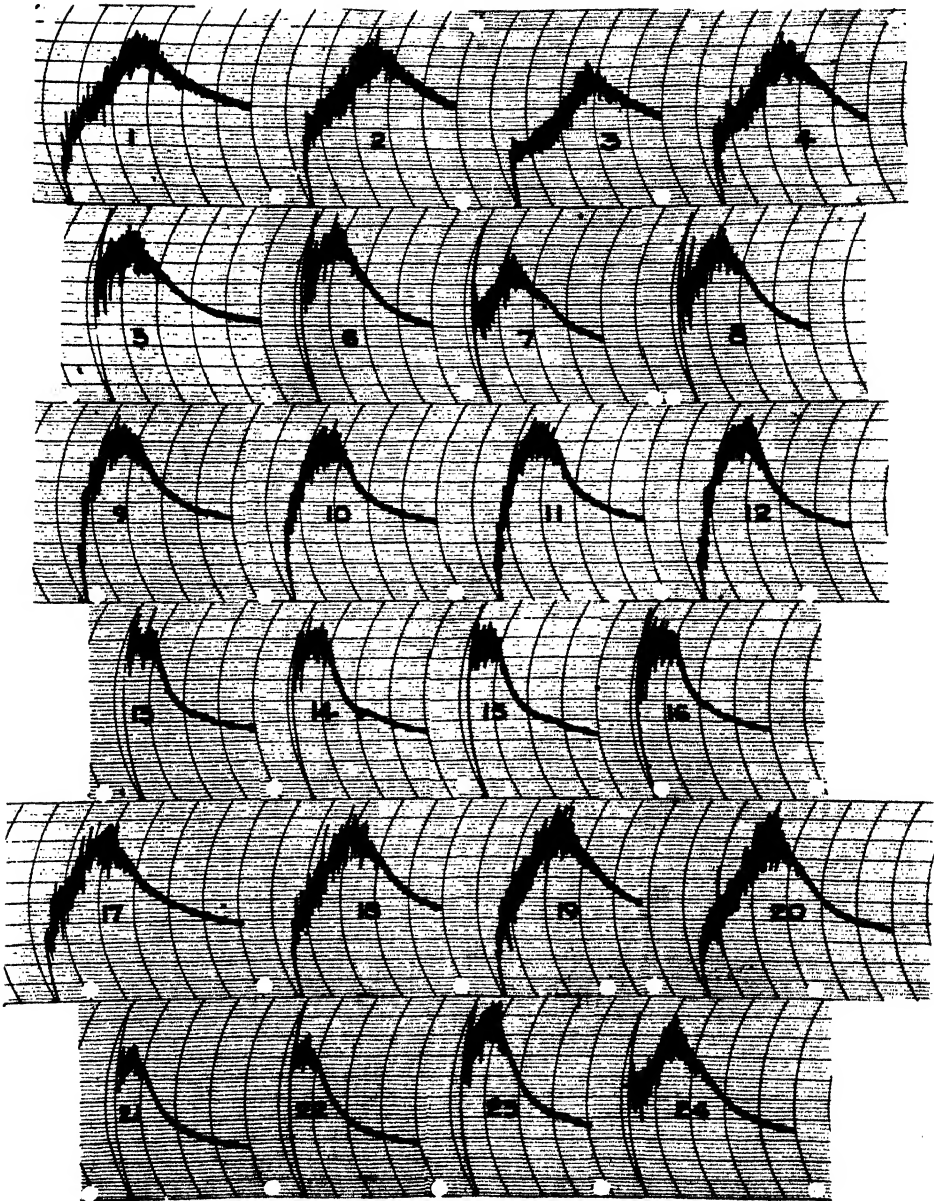


Fig. 3. Effects of  $\text{KBrO}_3$  on pepsin and papain.

		Amounts of $\text{KBrO}_3$			
		12 mg.	24 mg.	36 mg.	72 mg.
<i>Turkey + 12 mg. Pepsin</i>					
No incubation	(1)	(2)	(3)	(4)	
10 min. incubation	(5)	(6)	(7)	(8)	
<i>Chieftan + 12 mg. Pepsin</i>					
No incubation	(9)	(10)	(11)	(12)	
10 min. incubation	(13)	(14)	(15)	(16)	
<i>Turkey + 12 mg. Papain</i>					
No incubation	(17)	(18)	(19)	(20)	
10 min. incubation	(21)	(22)	(23)	(24)	

Any deviation is evidently due to the salt effects of  $\text{KBrO}_3$ . The second row shows the additional effect of the 10-minute incubation period. The upslope of these curves is greater than those in the second row of Figure 1, showing that the presence of  $\text{KBrO}_3$  had some effect on the curve characteristics. This difference is mostly in the first part of the curves which may be due to salt effects as shown in the preceding paper (Swanson, 1940b). The last part of these curves shows as much mobility as the curves in Figure 1 obtained in the absence of bromate. There were clearly no inhibiting effects on the increase of mobility in the last part of the curve.

The curves in the third and fourth rows of Figure 3 were made with Chiefkan flour under the same conditions as those of Turkey shown in the first and second rows. The effects of the pepsin and the  $\text{KBrO}_3$  on Chiefkan are no different from the effects on Turkey except in so far as the Chiefkan curves are different from the Turkey curves without any additions.

The curves in the fifth and sixth rows were made from Turkey, but using papain instead of pepsin. The greatest effect in increased mobility was obtained with the smaller amounts of  $\text{KBrO}_3$ . When 36 and 72 mg. of  $\text{KBrO}_3$  were used with no incubation period, there was a slower rate in the decrease of mobility, and with the incubation period there was considerable resistance shown on the upslope of the curve. The down slope, however, shows great increase in mobility; hence it is doubtful whether the  $\text{KBrO}_3$  by itself exercised any effects on the proteases used.

Bleaching with chlorine was tried as an inhibitor to the action of papain and cysteine on the curve characteristics. Doughs were made with Turkey flour bleached both at the rate of  $\frac{3}{4}$  and  $1\frac{1}{2}$  ounces of chlorine per barrel and using 3 mg. of cysteine and 12 mg. of papain with subsequent treatment as in the preceding curves. No pronounced differences could be observed in the characteristics of the curves made from the bleached flours as compared with those made from the unbleached flours. This is contrary to the findings of Balls and Hale (1936b), who reported that the time of sinking of BB shot into the dough was greatly increased as a result of bleaching with chlorine.

### Effects of Diastase and Taka-diastase

The diastases should influence the curve characteristics by their effects on the starch, which is present in the dough in six or seven times the amount of protein or gluten. This should be especially true of taka-diastase because of the alleged presence of the starch liquefying alpha-amylase. After several trials it was found that amounts larger than 12 mg. per 35 grams flour served no useful

purpose. The enzymes were added in water solution in order to be thoroughly incorporated with the dough. The curves obtained using the two diastases and the three flours are shown in Figure 4. The first curves in each row are the checks or those completed without any rest or incubation period. The middle curves in each row are those made after one hour's rest or incubation, and should show any enzyme effects exerted during the hour. The last curves in each row show the effect of one hour's incubation without the enzyme.

The first curves in each row are similar to those obtained with only flour and water. A comparison of the middle curves with the last curves in each row shows that autolysis in the absence of the diastases produced as great an effect as when the diastases were present. Further, the curves obtained with taka-diastase are but very little different from those produced with the diastase. Swanson (1940a) found that the diastases had no influence on the results of the wheat-meal-time-fermentation test while the proteases and protease activators, as well as the protease inhibitors, had a marked influence (Swanson and Dines, 1939). Read and Haas (1936), however, found that taka-diastase exhibited marked proteolytic activity. No evidence of this was found in these curves when taka-diastase was present.

### Discussion

The effects of the proteases, pepsin, trypsin, and papain on curve characteristics have been presented in this study. The extract of commercial wheat germ was used for comparison. Cysteine monohydrochloride was used as a protease activator and  $\text{KBrO}_3$  as a protease inhibitor. Diastase and taka-diastase were used to determine whether their action on starch had any influence on curve characteristics.

The proteases produced marked changes in the curves. These changes were similar to those produced by autolysis or incubating the flour and water dough one hour or more. The main difference was in time. The proteases produced in a 10-minute incubation period as much effect as one to several hours of incubation without the protease. Papain produced the greatest effect, pepsin the least, and trypsin produced an intermediate effect.

Two minutes of contact with cysteine, including both the mixing and resting time, produced as great a change as ten minutes with the proteases or several hours' incubation of the plain water-and-flour dough. From the previously quoted statements of Balls and Hale (1936b) as well as these results it would appear that cysteine activates both the protein and the proteases. Without such a supposition it is difficult to account for the rapid action of the cysteine.

As a representative protease inhibitor,  $\text{KBrO}_3$  was used in 12, 24,

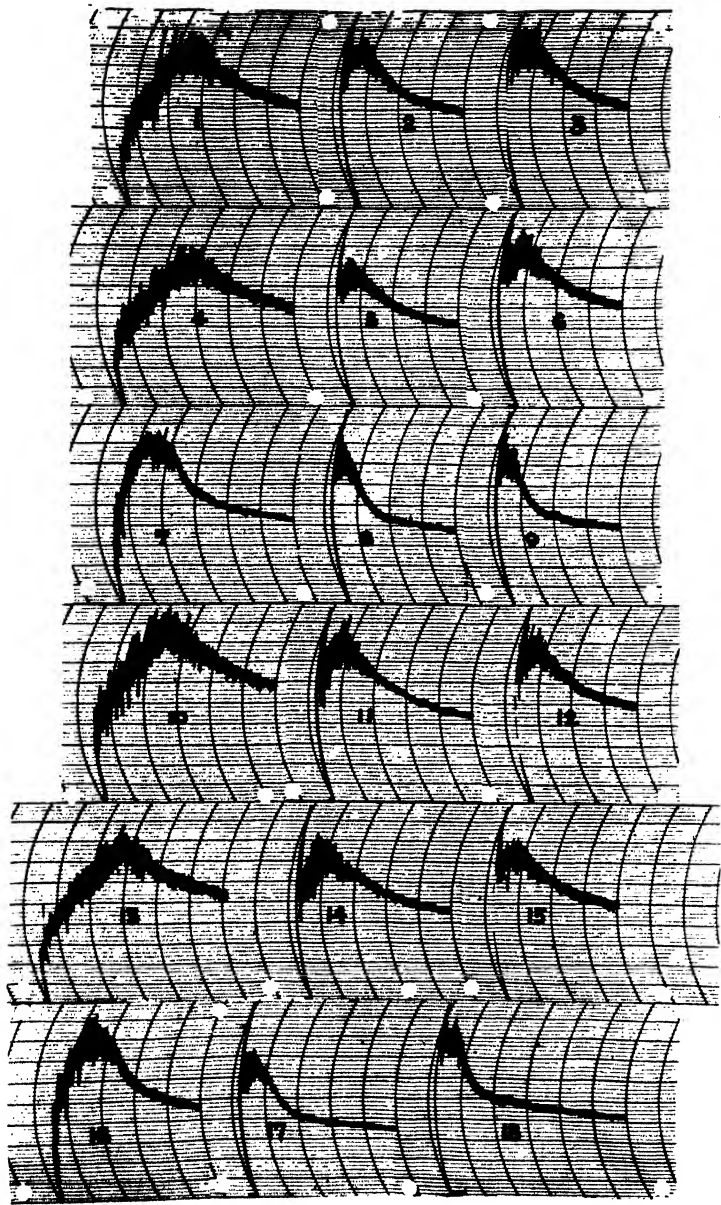


Fig. 4. Effects of diastase and taka-diastase.

	12 mg., no incubation	12 mg., 1 hr. incubation	No mg., 1 hr. incubation
	<i>Diastase</i>		
Turkey	(1)	(2)	(3)
Tenmarq	(4)	(5)	(6)
Chiefkan	(7)	(8)	(9)
	<i>Taka-diastase</i>		
Turkey	(10)	(11)	(12)
Tenmarq	(13)	(14)	(15)
Chiefkan	(16)	(17)	(18)

36, and 72 mg. amounts for each dough made with 35 g. of flour. From the characteristics of the curves it could not be shown that the  $\text{KBrO}_3$  had any inhibiting effect. Salts have by themselves a stiffening effect on the gluten, and hence it is not possible to determine definitely whether the decrease in mobility was due to the inhibiting action of the  $\text{KBrO}_3$  on the proteases or whether it was due to a salt effect.

The presence of diastase and taka-diastase produced no results essentially different from autolysis without these enzymes. Hence, their effects on starch were not noticeable in the curve characteristics.

### Conclusion

The effects on curve characteristics of incubating flour-and-water doughs are very similar to the effects produced by proteases and the protease activator, cysteine, except as regards the time necessary for effects to become evident. From this it may be inferred that the influence of autolysis on curves can be attributed to proteases or protease activators.

### Literature Cited

- Balls, A. K., and Hale, W. S.  
1936a Proteolytic enzymes of flour. *Cereal Chem.* **13**: 54-60.  
1936b Further studies on the activity of proteinase in flour. *Cereal Chem.* **13**: 656-664.
- Flohil, J. T.  
1936 The effect of chemical flour improvers on proteolytic action in relation to the gas-retaining capacity of fermenting doughs. *Cereal Chem.* **13**: 675-689.
- Hopkins, F. G.  
1929 On glutathione: a reinvestigation. *J. Biol. Chem.* **84**: 269.
- Jørgensen, H.  
1936 On the existence of powerful but latent proteolytic enzymes in wheat flour. *Cereal Chem.* **13**: 346-355.
- Read, J. W., and Haas, L. W.  
1936 Further studies on the baking quality of flour as affected by certain enzyme actions. *Cereal Chem.* **13**: 14-37.  
1937a Baking quality of flour as affected by certain enzyme actions. III. Purified amylase and the relative proteolytic activity of amylolytic agents. *Cereal Chem.* **14**: 58-73.  
1937b Studies on the baking quality of flour as affected by certain enzyme actions. IV. Potassium bromate and enzyme activity. *Cereal Chem.* **14**: 752-766.  
1939 Studies on the baking quality of flour as affected by enzyme actions. VI. Further studies relating to the activation and the inhibition of flour proteinase. *Cereal Chem.* **16**: 60-70.
- Sullivan, B., Howe, M., and Schmalz, F. D.  
1936 On the presence of glutathione in wheat germ. *Cereal Chem.* **13**: 665-669.
- Swanson, C. O.  
1940a The wheat-meal-time-fermentation test. IV. Inhibitory and accelerating factors. *Cereal Chem.* **17**: 355-372.  
1940b Factors which influence the physical properties of doughs. I. Effects of autolysis on the characteristics of dough mixer curves. *Cereal Chem.* **17**: 679-689.
- Swanson, C. O., and Dines, F. T.  
1939 The wheat-meal-time-fermentation test. II. Effects of proteases, protease activators, and protease inhibitors. *Cereal Chem.* **16**: 168-177.

# A METHOD FOR THE FORMULIZATION OF FARINOGRAPH CURVES

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(Received for publication March 7, 1940)

Considerable controversy has existed as to the best method for recording and interpreting curves produced by the Brabender farinograph and several different techniques have been suggested for the characterization of these data. However, in the course of a summer's work at the National Milling Company, Toledo, Ohio, during which farinograph curves were made for all incoming lots of grain, it was decided that the existing methods for the interpretation of these curves do not provide an adequate means for the expression of curve characteristics. The usual procedure is to consider only the time during which the upper line of the graph stays above a certain ordinate. This is very arbitrary and empirical and it yields only one item of data from the curve, whereas the curve may be capable of expressing much more. Furthermore, in accordance with the usual type of procedure, a true and complete picture of the curve would require many measurements.

This situation led to the thought that if the farinograph curve could be represented by a formula in which the consistency in Brabender units, as well as the mixing time, were given explicitly as functions of certain arbitrary constants, then all of the information contained in the curve would be available in compact mathematical form. This formula must, of course, be such a combination of consistency, time, and arbitrary constants that every farinograph curve could be reproduced by adjustments of the constants only.

With such a formula it is a simple mathematical manipulation to obtain from it any feature which the original curve contained, and the formula serves as a simple, compact evaluation of the original curve obtained from the farinograph. Also it is at least possible that correlations may be found between the constants of the formula and certain flour properties such as baking characteristics, protein, or other quality factors. With these aims in mind a method is herein proposed.

## The Method

In obtaining a formula which is to fit the original farinograph curve, the formula must be such that when, using farinograph paper, values of time on the one hand are plotted against values for consistency on the other, a close approximation of the original curve will be reproduced.



Accordingly, by intuition and trial, the writer obtained the following formula which fits any farinograph curve, by adjustment of the arbitrary constants  $A$ ,  $n$ , and  $c$  only:

$$K = At^n e^{-ct}$$

in which:  $t$  is the time in minutes,

$K$  is the consistency in Brabender units,

$e$  is the base of Napierian logarithms,

$A$ ,  $n$ , and  $c$  are the arbitrary constants adjusted to fit the curve in question.

It was found by numerous trials that this formula will accurately represent any farinograph curve from zero time up to a point considerably beyond gluten breakdown. Since the Brabender farinograph curves give no information at points far beyond the breaking point of the gluten, the formula fulfills its mission in representing the curve over its entire useful range.

Let us consider the theoretical aspects of the problem of fitting this formula to any given curve. Since there are three unknowns in the equation to be evaluated, namely the three arbitrary constants  $A$ ,  $n$ , and  $c$ , it will be necessary to take three items of data from the original curve to solve for these unknowns. Thus, if we select three points on the curve in the region of interest, and substitute the coordinates of each of these in turn into the aforementioned equation, we will obtain three equations in which the only unknowns are the three arbitrary constants  $A$ ,  $n$ , and  $c$ . It is well known that three independent equations of linear form with three unknowns may be solved to obtain the three unknowns by the simultaneous theory. However, by inspection it appears that the three equations are not linear since they contain an exponential; hence we take logarithms to the natural base of both sides of each of them, and obtain equations which are now linear in logarithms. The aforementioned equation appears as follows after logarithms to the natural base have been taken of both its sides:

$$\text{Log } K = \log A + n \log t - ct$$

in which all symbols are as before.

Solving the three linear equations simultaneously and obtaining the three constants  $A$ ,  $n$ , and  $c$  in their numerical form for the particular curve, we can then substitute them in the original general equation, and the formula of the special curve in question has been obtained.

In passing it may be mentioned that the three points from which data are taken may be somewhere near the top of the curve, in the region in which baking is likely to be done, since the formula auto-

matically reduces the consistency to zero at zero time and thus actually supplies another point on the curve in addition to the three chosen by the operator. The writer has arbitrarily selected the point of maximum consistency, the point at which the time is half that observed at the maximum consistency, and the point three-quarters of the distance between the time observed at maximum consistency and that observed at the point where the top of the curve crosses the line about which the curve has been centered ("600" line). All of these points are taken on the top of the curve for uniformity although they might just as well be taken in the middle or at the bottom of the curve. These locations are purely arbitrary and are used to provide uniformity of procedure in order that the results of many trials may be reasonably compared. It is possible that further systematic investigations of this process may modify these methods.

The following example will illustrate the procedure which has been outlined. Figure 1 shows a typical farinograph curve selected at

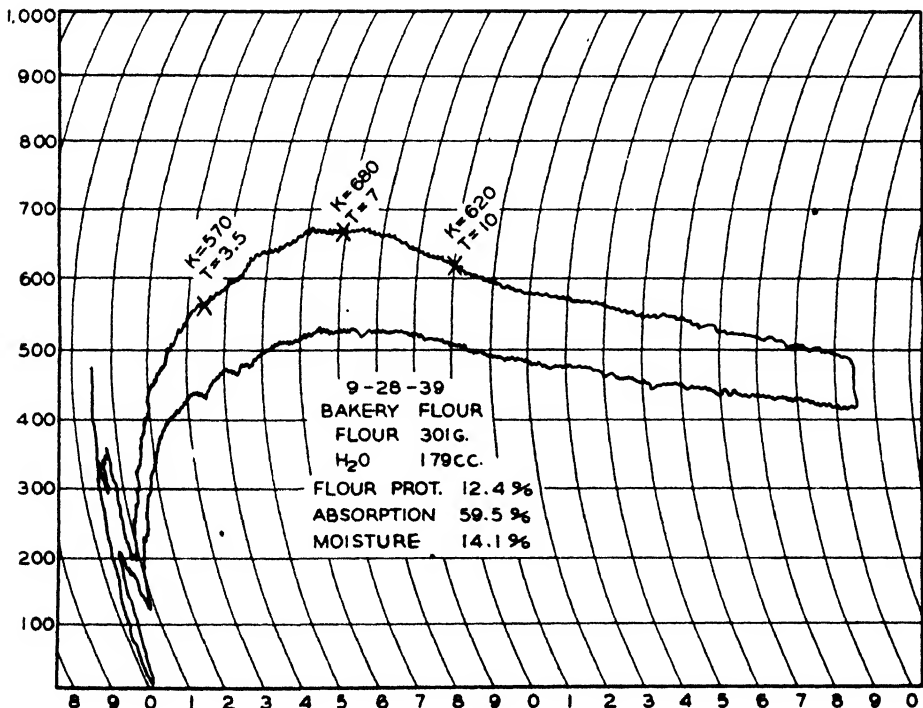


Fig. 1. Formulation of a typical farinogram.

random. It typifies the curve of a bakery flour made by the National Milling Company. Three points are selected, using the procedure above outlined, as indicated by the cross marks on the top of the curve. The coordinates of these points are marked at each point. Substituting

these points in the general equation we have as follows, three equations:

$$\begin{aligned} 570 &= A(3.5)^n e^{-3.5c} \\ 680 &= A(7.0)^n e^{-7.0c} \\ 620 &= A(10.0)^n e^{-10.0c}. \end{aligned}$$

Taking the logarithms to the natural, or Napierian base, of both sides of these equations we have:

$$\begin{aligned} \text{Log } 570 &= \log A + n \log 3.5 - 3.5c \\ \text{Log } 680 &= \log A + n \log 7.0 - 7.0c \\ \text{Log } 620 &= \log A + n \log 10.0 - 10.0c. \end{aligned}$$

Looking up these logarithms in tables and substituting their numerical values, we have:

$$\begin{aligned} 6.345 &= \log A + 1.253n - 3.5c \\ 6.522 &= \log A + 1.946n - 7.0c \\ 6.430 &= \log A + 2.303n - 10.0c. \end{aligned}$$

Solving these equations simultaneously we have:

$$\begin{aligned} \text{Log } A &= 5.610 \text{ or } A = 272.0 \text{ (obtained by looking up the number} \\ n &= 1.02 \text{ whose log is 5.610)} \\ c &= .153 \end{aligned}$$

Having solved these equations for their unknowns we can now place the unknowns in the original equation and we have:

$$K = 272(t)^{1.0} e^{-.153t},$$

which is the formula for the farinograph curve just considered.

In order to determine how closely this equation fits the actual curve we can make a table of the time and consistency at several different intervals as observed from the original curve, and compare these consistencies thus observed with the values obtained by substituting these same times or intervals in the above formula. If the agreement between these values is close, we can assume that the method has proved satisfactory in this case. The results are given in Table I.

From this table it may be seen that the calculated results were, in all cases, within twenty Brabender Consistency Units of the observed values, indicating an accuracy of about 5%, which is closer than the curve can be read for most purposes. Furthermore the formula is seen to be accurate beyond the last point for which it was calculated, namely  $t$  equals ten.

TABLE I  
OBSERVED AND CALCULATED CONSISTENCIES

Time (min.)	Consistency (observed)	Consistency (calculated)
0	0	0
2	430	410
4	590	592
6	670	654
8	670	663
10	620	620
12	580	563

The results obtained here are typical of the results obtained in all cases in which the formula has been applied, showing its reliability.

Assuming accuracy and reliability of the formula, the question as to its value and application may be considered.

In this connection two selected lots of flour were mixed in different graduated proportions as indicated in Table II. The farinograph curve for each mixture was obtained and the formulation of each curve was accomplished. The results are given in Table II.

In Table II the whole formula has not been given but only the necessary constants are tabulated.

From an inspection of the results it appears that there is a definite correlation between the mixtures and the arbitrary constants. The figures all proceed one way with few exceptions, and these exceptions may be explained perhaps by the fact that the data are so arbitrarily taken. It has been found that a flour which retains its strength for a long period has low values of its constants and that a high *A* constant is indicative of a rapid rise of consistency.

The writer can only suggest that correlations may exist between these arbitrary constants and baking or chemical characteristics of the flour.

### Precision of Method

From long experience it has been found that the precision is always within 5%, which is possibly greater than the reproducibility of the farinograph curves themselves. This corresponds to an accuracy of about 20 Brabender Consistency Units, or one of the smallest squares on the standard farinograph paper.

### Summary and Conclusions

It has been found possible to represent the outlines of farinograph curves accurately by means of a mathematical formula in which there are three arbitrary constants which by adjustment will allow the formula to be fitted with high precision to any farinograph curve.

TABLE II  
CALCULATED CURVE CONSTANTS

Flour Lot No. 1		<i>A</i>	<i>n</i>	<i>c</i>
Flour A	Flour B			
%	%			
100	0	487	0.268	0.0396
90	10	451	0.366	0.0468
80	20	379	0.590	0.0765
70	30	512	0.392	0.0653
60	40	485	0.547	0.0915
50	50	566	0.291	0.0549
40	60	576	0.395	0.0936
30	70	653	0.592	0.201
20	80	686	0.739	0.258
10	90	663	0.990	0.382
0	100	906	1.40	0.615

Flour Lot No. 2		<i>A</i>	<i>n</i>	<i>c</i>
Flour C	Flour D			
%	%			
100	0	605	0.145	0.027
90	10	570	0.307	0.0595
80	20	554	0.465	0.0953
70	30	567	0.370	0.0845
60	40	556	0.505	0.1080
50	50	604	0.540	0.1560
40	60	628	0.781	0.261
30	70	742	0.842	0.250
20	80	824	1.08	0.471
10	90	896	0.760	0.402
0	100	940	1.42	0.633

The constants obtained from systematic investigations show variations proportional to the variations of the curves themselves, and probably indicate different characteristics of the flour and wheat. It is suggested that this new method of evaluating the farinograph curves offers a reliable means for characterization, while at the same time incorporating all useful data obtainable from the curves.

It is suggested that the use of this formula for predicting the consistency for any given mixing time would permit the construction of tables by which the miller and baker would have a practical working guide as to the proper mixing time, and would provide as suitable criteria of baking properties as would the curve itself.

It is anticipated that the cereal chemists will find sufficient interest in this method to correlate the arbitrary constants with characteristics of the flour, wheat, or the finished product in order to make the formula useful in predicting as well as in shortening the notation of farinograph curves.

# AN APPARENT RELATIONSHIP BETWEEN PROTEIN CONTENT AND RATE OF PROOF<sup>1</sup>

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(Read at the Annual Meeting, May 1940)

There is little doubt that the two main factors involved in the production of bread from flour are (1) enough sugar to produce sufficient gas throughout the entire dough-fermentation period and (2) the ability of the flour dough to retain the gas produced.

It is also true that gas production during the latter stages of fermentation is dependent principally upon the fermentation of maltose produced by diastatic action. Sandstedt and Blish (1934) determined the presence of some substance or substances in flour which have a stimulating effect upon the fermentation of maltose and which they designated as "activators." Blish and Sandstedt (1937) made a study of the properties of this "activator" and determined that it occurs in varying amounts in different flours. They found that malted wheat flours contained significantly higher quantities of this material than did normal flours. They were unable to identify this "activator" but tentatively designated it as "factor M." Sandstedt and Blish (1938) devised a means for estimating the "activator content" of flours by the manometric fermentation technique. A series of 33 flours was observed in baking in an attempt to correlate "activator content" with the time required to proof to a standard height under conditions affording adequate sugar levels in all doughs. Considerable differences in the rate of proof were found, even though the sugar levels were adequate. The conclusion from these results was that in experimental baking investigations proofing should be to a definite height rather than for a constant period of time.

The object of the present paper is to present further evidence (obtained during a study of a number of wheat varieties) which supports the above conclusion.

## Methods and Materials

The varietal series of flours used in this investigation were obtained through the courtesy of E. G. Bayfield and the Department of Milling Industry at Kansas State College. They were grown and composited in a manner identical with the series used by Larmour, Working, and Ofelt (1939) in their study of hard winter wheat quality.

<sup>1</sup> Journal Series No. 266, Nebraska Agricultural Experiment Station. Printed with the approval of the Director.

The samples of irrigated Cheyenne and Supreme were obtained from W. O. Whitcomb of the Montana Agricultural Experiment Station. Those of Dawson and Trumbull were obtained from the Federal Soft Wheat Laboratory at Wooster, Ohio. Those of Turkey and Kawvale-Tenmarq were grown in test plots at the Nebraska Agricultural Experimental Station.

The baking formula No. 1 used when proof height was determined was the same as that used by Larmour, Working, and Ofelt (1939) and the procedure the same with the exception that "micro" methods were used. Doughs were based on 25.0 g. of flour (15% moisture basis) and the loaves were baked in pans which were scaled down from the "Markley type" or low form pan. A constant proof time of 55 minutes was used.

The formula used when proof time was determined (No. 2) was as follows: sugar 6%, salt 1%, yeast 3%, shortening 3%, malt flour 0.5%, dry milk solids 6%, and potassium bromate 0.004%. The doughs were proofed to a constant height of 6.1 cm. in pans scaled down from the standard high-form "pup" type pans.

Hydrolyzed gluten, added in certain experiments, was obtained by hydrolyzing 10 g. of ball-milled dried gluten with 1 g. of papain and 1 g. of trypsin for a period of two hours at 30° C. and then boiling for a period of at least twenty minutes to destroy the enzymes.

### Experimental

The variety-protein series was baked using formula No. 1. The height of the loaves was measured at the end of the proof period of 55 minutes, at which time the loaves went to the oven. These data are given in Table I. The same series was then baked using formula No. 2 and the loaves were proofed to a standard height of 6.1 cm. The time required to reach this definite height is also recorded in Table I. These data show that when proofing to time there is a definite increase in proof height at increasingly higher protein levels. When proofing to height, the decrease in proof time with increasing protein content is even more striking.

It becomes obvious after an examination of the data in Table I that either the gas retention or the rate of gas production during the proofing period is greater for the high-protein flours than for the lower-protein flours.

The gas-retaining properties of doughs are difficult to determine. Gas production is determined easily and conveniently. Accordingly the latter factor was chosen for study. "Activator content," or third-hour gas production with the flour as the only variable, was determined by

TABLE I

RELATION BETWEEN PROOF HEIGHT AND PROTEIN CONTENT WHEN PROOFING TO TIME AND THE RELATION BETWEEN PROOF TIME AND PROTEIN CONTENT WHEN PROOFING TO A DEFINITE HEIGHT

Per cent protein	Proof height (55 min.)	Proof time (to 6.1 cm.)	Percent protein	Proof height (55 min.)	Proof time (to 6.1 cm.)
BLACKHULL			CHIEFKAN		
10.0	4.8	47	10.5	4.9	51
11.0	4.8	43	11.1	4.9	51
12.5	5.1	38	12.6	5.1	48
14.3	5.1	35	14.0	5.4	40
15.5	5.3	33	15.1	5.4	39
16.5	5.3	33	16.1	5.5	39
TENMARQ			TURKEY		
9.8	4.8	47	8.7	4.7	47
10.9	4.8	47	9.4	4.7	46
11.1	4.8	45	10.7	4.9	45
12.3	5.1	43	12.6	5.2	41
13.7	5.4	35	13.7	5.4	37
15.4	5.5	33	15.3	5.4	34
16.9	5.5	29	16.1	5.5	31

the Sandstedt-Blish (1938) method on all samples of each of the variety-protein series. The results on four of the varieties are graphically represented in Figure 1. These results show clearly that there is a definite and nearly linear increase in *rate* of gas production during the critical proofing period with increase in protein content. The writers believe that the data in Figure 1 are sufficient evidence to indicate that the only present means of eliminating the variable of gas-production rate during the critical proofing period is to proof to a constant height.

Schultz, Atkin, and Frey (1939) have indicated that differences in the third-hour fermentation rate between various flours may be due to differences in their amino-nitrogen content. They determined the effect of 22 amino acids on the third-hour fermentation rate of a patent flour using a yeast-flour-water-maltose mixture. The greatest increase in third-hour gassing rate was produced by the addition of *l*-asparagine. It was found in the course of the present investigation that the maximum effect could be obtained with 25 mg. of asparagine per 10 g. of flour. An attempt was made to bring three flours to a common level of third-hour gas production by the addition of asparagine. The results are given in Table II. It is evident that the addition of asparagine did not bring all flours to a common level of third-hour gas production.

Addition of ball-milled gluten had no effect upon the rate of gas production but the addition of the same amount of this gluten after



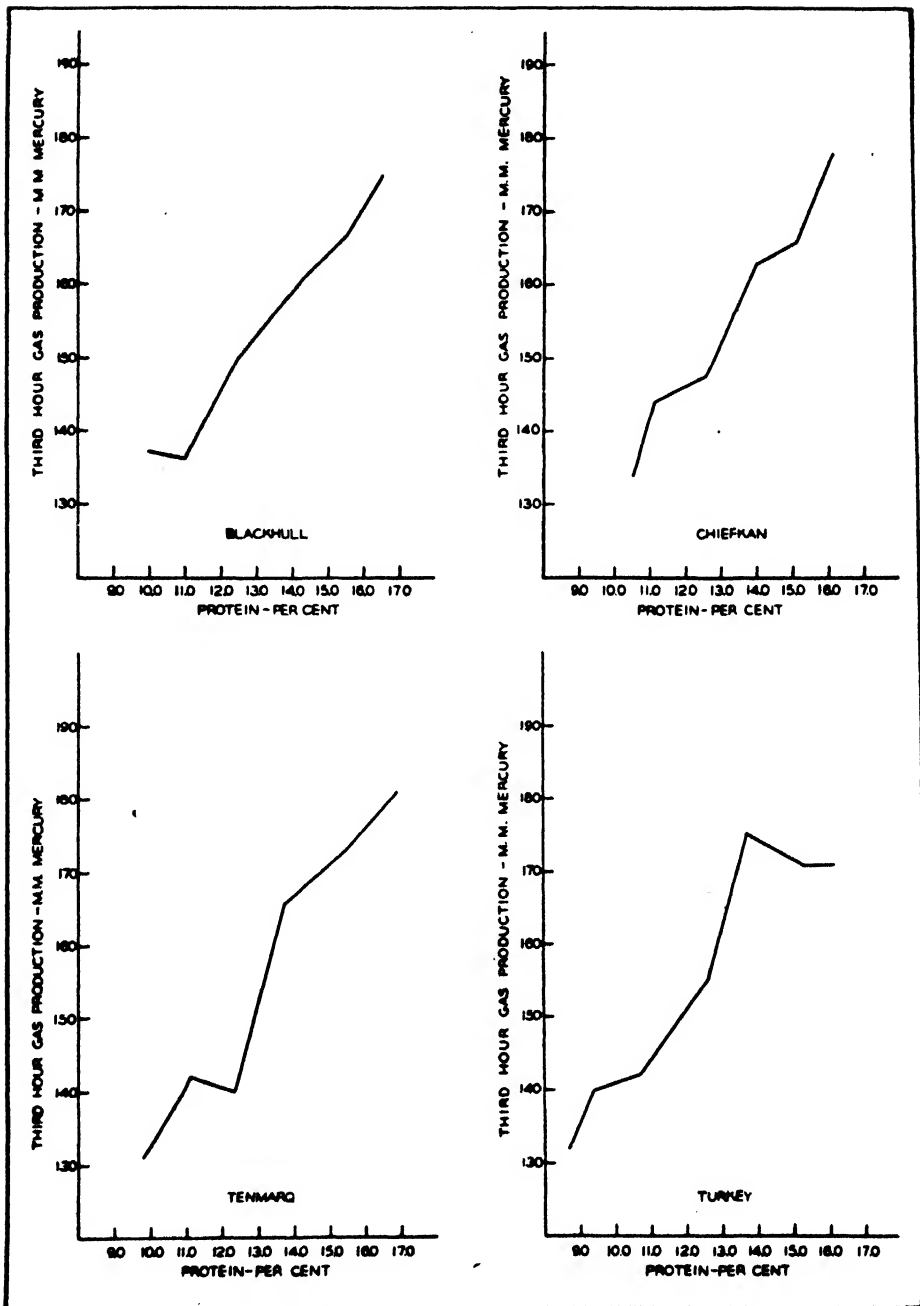


Fig. 1. The relationship between protein content and rate of third-hour gas production.

hydrolysis with papain and trypsin had a very definite effect on the rate of gas production. It was determined that the maximum effect could be obtained by the addition of 300 mg. of the hydrolyzed gluten as shown in Table III. The data in Table IV show that flours obtained

from different varieties of wheat and of widely differing protein contents can be brought to a nearly uniform level of gas production during the critical third hour of fermentation or the proofing period.

Several flours were baked with and without the addition of hydrolyzed gluten in an effort to determine the practicability of the procedure in

TABLE II  
EFFECT OF ADDITION OF *l*-ASPARAGINE ON THIRD-HOUR GAS PRODUCTION

Flour	Asparagine added (mg.)	Third-hour gas production (mm. Hg)
Nebr. No. 1	0	157
No. 1	25	185
No. 20	0	143
No. 20	25	193
No. 21	0	153
No. 21	25	176

TABLE III  
EFFECTS OF VARYING AMOUNTS OF HYDROLYZED GLUTEN  
ON THIRD-HOUR GAS PRODUCTION

Flour	Hydrolyzed gluten added (mg.)	Third-hour gas production (mm. Hg)
S. W. Composite	0	174
S. W. Composite	50	203
S. W. Composite	100	223
S. W. Composite	200	228
S. W. Composite	300	231
S. W. Composite	400	233
S. W. Composite	500	233
No. 20	0	140
No. 20	50	178
No. 20	100	204
No. 20	200	207
No. 20	300	212
No. 20	500	209

establishing a common proof time for all flours, but the hydrolyzed gluten had too great an effect upon the dough properties to allow for its practical application.

Since ammonium salts are also known to affect the rate of fermentation, monoammonium phosphate and ammonium chloride were added to flours of widely differing type and "activator content." Data given in Table V show the effects of the monoammonium phosphate on the

TABLE IV

EFFECT OF HYDROLYZED GLUTEN ON THIRD-HOUR GAS PRODUCTION WHEN ADDED TO FLOURS WHICH ORIGINALLY HAVE A WIDE RANGE IN RATE OF GAS PRODUCTION

Variety	Percent protein	Third-hour gas production (mm. Hg)	Third-hour gas production (mm. Hg) with hydrolyzed gluten added
Blackhull	14.3	161	208
Blackhull	15.5	167	214
Blackhull	16.5	175	211
Early Blackhull	15.6	167	213
Chiefkan	14.0	163	211
Chiefkan	15.1	166	208
Chiefkan	16.1	178	216
Cheyenne	10.1	133	209
Cheyenne	13.7	167	208
Kanred	13.9	166	211
Kanred	16.4	173	208
Iobred	13.7	144	200
Tenmarq	12.3	140	196
Tenmarq	15.4	173	204
Turkey	10.7	142	201
Turkey	16.1	171	208

TABLE V

EFFECT OF 0.5%  $\text{NH}_4\text{H}_2\text{PO}_4$  ON THIRD-HOUR GAS PRODUCTION AND PROOF TIME WHEN ADDED TO FLOURS WHICH DIFFER WIDELY IN TYPE AND PROTEIN CONTENT

Flour	Percent protein (15% m.b.)	Normal third-hour gas production (mm. Hg)	Third-hour gas production (mm. Hg) with 0.5% $\text{NH}_4\text{H}_2\text{PO}_4$	Normal proof time	Proof time with 0.5% $\text{NH}_4\text{H}_2\text{PO}_4$
Irrigated Cheyenne	7.2	111	196	68	45
Irrigated Cheyenne	10.1	142	199	49	36
Irrigated Supreme	8.3	126	186	54	41
Irrigated Supreme	9.8	143	193	56	41
Kawvale $\times$ Tenmarq	13.3	167	192	36	31
S. D. Turkey	14.8	166	194	36	30
Nebr. Turkey	14.7	169	189	34	32
Dawson	7.9	140	195	57	43
Trumbull	9.0	145	197	53	43

rate of gas production during the third hour and also the effect on proof time when these same flours were baked and proofed to a definite height. The data show that, even with flours differing as widely as these in type and protein content, third-hour gas production can be brought surprisingly near to a uniform rate. It is very evident that it is not possible to bring these flours to a uniform proofing time, although

the spread of proof times was reduced by one-half. Undoubtedly this was because of extreme differences in gas-retention properties of doughs from these flours. The possibility still remains that the differences in gas-retention properties among normal hard wheat flours may not be too great to allow all to be brought to a uniform proofing time by the addition of these ammonium salts.

### Summary and Conclusions

A number of flours were baked by two methods and it was established that with increasingly higher protein levels (1) proof height increased when proofing to time and (2) proof time decreased when proofing to height.

The rate of gas production during the third hour was determined on the same samples and was found to increase in almost linear relationship with increasing protein content. Proofing to a definite height is the only present means of decreasing if not eliminating the effects of this variable.

It was established that flours could not be brought to a common level of third-hour gas production by the addition of *l*-asparagine.

Hydrolyzed gluten was added to a number of samples of widely differing protein and "activator" contents and it was shown that this brought all flours to a nearly uniform rate of third-hour gas production. This procedure was, however, found to be impracticable in baking.

Ammonium salts were added to a number of flours and the third-hour gas production rate made nearly uniform. Proof time for these flours was not brought to a common level by this procedure although the range of proof times was reduced by one-half.

### Literature Cited

- Blish, M. J., and Sandstedt, R. M.  
1937 Biocatalytic activators specific for the yeast fermentation of maltose. *J. Biol. Chem.* **118** (No. 3): 765-780.
- Larmour, R. K., Working, E. B., and Ofelt, C. W.  
1939 Quality tests on hard red winter wheats. *Cereal Chem.* **16**: 733-752.
- Sandstedt, R. M., and Blish, M. J.  
1934 Yeast variability and its control in flour gassing power tests. *Cereal Chem.* **11**: 368-383.
- 1938 Maltose fermentation activators as affecting baking. *Cereal Chem.* **15**: 788-794.
- Schultz, A. S., Atkin, L., and Frey, C. N.  
1939 Fermentation of maltose in the dough. *Cereal Chem.* **16**: 648-651.

# A VARIETAL STUDY OF THE RELATION BETWEEN PROTEIN QUALITY AND PROTEIN CONTENT <sup>1</sup>

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(Read at the Annual Meeting, May 1940)

Comparison of baking qualities of flours of widely varying protein content has always been exceedingly difficult. The chemist, however, is frequently required to make such comparisons. The agronomist wishes to know the relative baking qualities of wheats grown under widely differing conditions, the effects of soil and climate upon protein quality as well as quantity, and whether the quality of a given variety is constant regardless of the protein quantity. The miller wants to know the relative blending values of wheats of differing protein content. Larmour, Working, and Ofelt (1939) propose as a measure of quality the divergence from expectation based on protein content; *i.e.*, the quality is judged by comparing the loaf volume obtained from the flour in question with a standard regression curve obtained from protein-volume values. This should give the relative quality of flours at any particular protein level; however, the difficulty of comparing the qualities of flours at differing protein levels still remains.

Sandstedt, Jolitz, and Blish (1939) have shown that the gluten and starch which have been separated by washing gluten can be recombined to form a reconstituted dough which has baking properties similar to dough made from the original flour. It would then seem reasonable to suppose that wheat starch could be added to a high-protein flour to produce a flour of any lower protein content which may be desired or conversely that gluten washed from a low-protein flour could be added to the original flour to produce a flour with any desired higher protein content. This might afford a method of comparing the baking qualities directly on a basis of a constant protein level. Such a method might also prove to be a means for determining blending quality.

If one accepts the suggestion that protein content and strength are synonymous (Blish and Sandstedt, 1935; Larmour, Working, and Ofelt, 1939) and that the deviation from the expected potentiality is a measure of quality, it would seem imperative that the maximum loaf volume be obtained in baking. Aitken and Geddes (1934) and Larmour, Working, and Ofelt (1939) have indicated the possibility of obtaining the maximum volume from a single bake using a formula which is optimum or nearly optimum for all flours. If this is possible it simplifies the

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<sup>1</sup> Journal Series No. 265, Nebraska Agricultural Experiment Station. Printed with the approval of the Director.

work of determining quality even though it does not simplify the determination of flour characteristics. The responses, tolerances, and requirements of a flour must still be determined by the use of supplementary procedures.

### Methods

The formulas proposed as optimum vary considerably and the question naturally arises as to their applicability to all flours. Accordingly, in studying the possibility of testing all flours on a definite protein basis, it was thought best to determine whether the absence or inclusion of some of the commonly used supplementary ingredients might affect the judgment of relative quality. It was decided to use the A. A. C. C. basic formula with 6% sugar with supplements of shortening (3%), malted wheat flour (0.5%), and dry milk solids (6%). The milk used was obtained from the same source as that used by Larmour, Working, and Ofelt (1939). Each of these formulas was further supplemented with 1, 2, and 4 mg.% of potassium bromate.

The micro baking technique, using 25 g. of flour, described by Van Scoyk (1939) was used through this work after Van Scoyk's claims for the method had been verified. All doughs were mixed to the so-called "optimum" in a National micromixer. This point was determined by observing the transition of the mixing dough from the characteristic rough appearance of the undermixed dough to the velvety smoothness of a well developed dough. The doughs were molded by passing through the National dough sheeter set at 5/32 inch and rolling up as lightly as possible by hand.

The following four experimentally milled, unbleached samples of flour were chosen for this investigation because of their differing yet representative characteristics:

Fulcaster,	9.9% protein.
Blackhull,	15.8% protein.
Tenmarq,	14.4% protein.
Nebred,	16.1% protein.

Each flour was baked undiluted and after dilution with starch (prepared by the method described by Sandstedt, Jolitz, and Blish, 1939) to a 10% protein level.

### Effect of Formulas on Determination of Quality

The results obtained on the original undiluted flours are shown in Figure 1. It is seen that for the milk-free formulas the maximum volume was obtained with 2 mg.% of bromate. The addition of milk

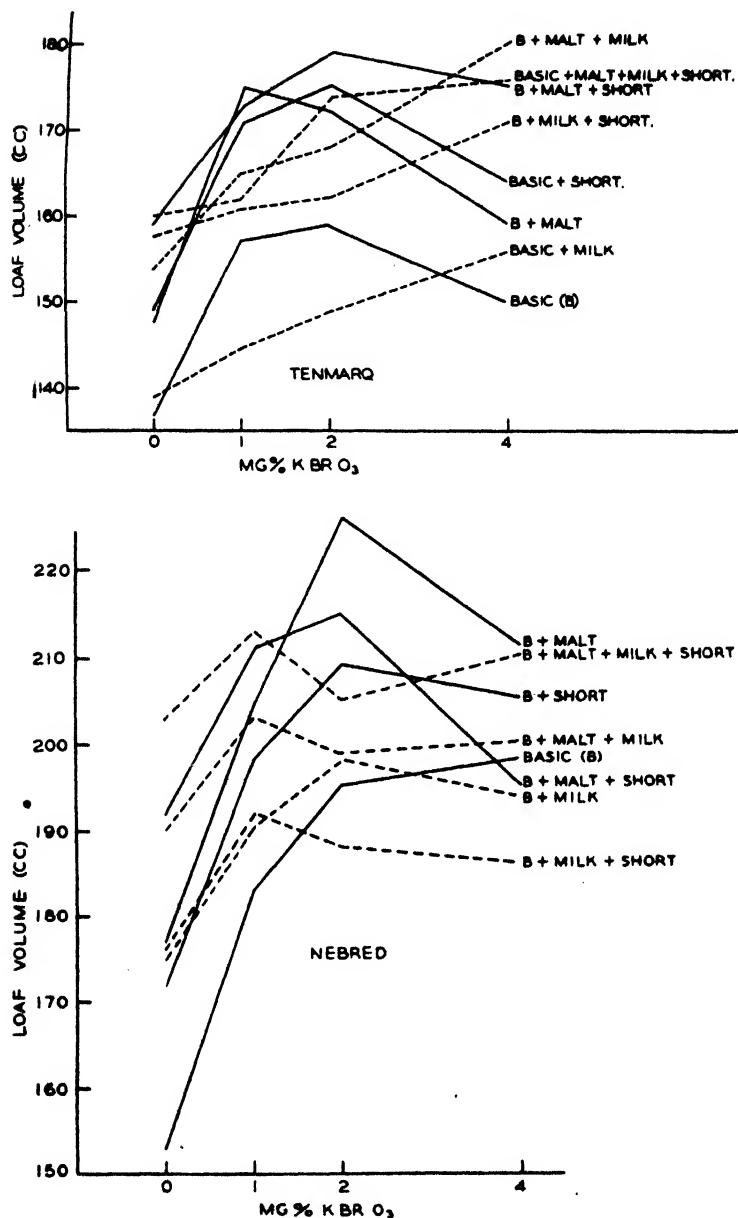
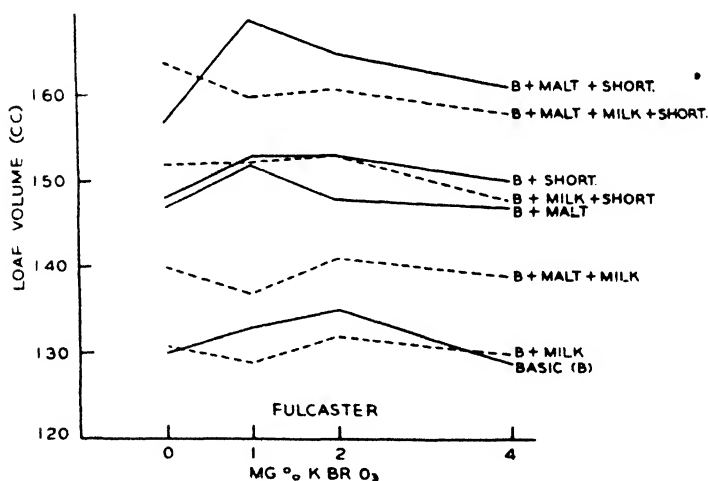
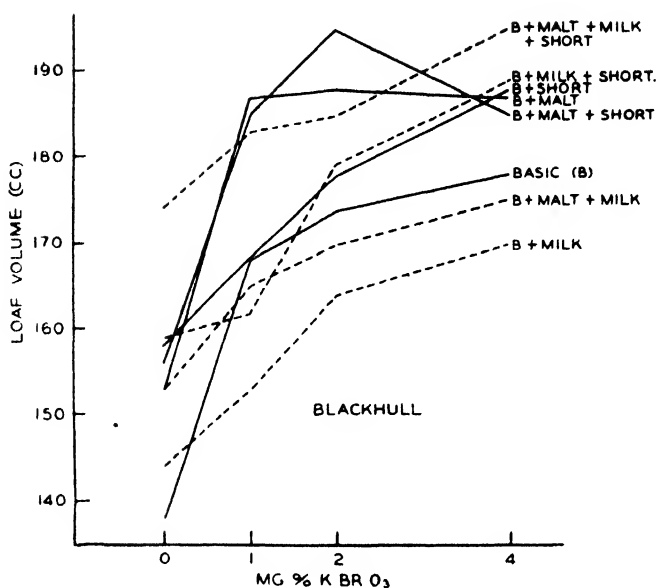


Fig. 1. Comparison of loaf volumes obtained by

to the formulas increased the bromate requirements for the Blackhull and Tenmarq samples to 4 mg.% (or more). Shortening and malt each increased the volume and when both were used the effect was roughly additive. The similarity between curves for the milk-free formulas for each variety indicates that the use of shortening and/or malt does not materially change the bromate requirements, responses, or tolerances.



variations in the baking formula on natural flours.

The results obtained by baking these flours at an artificially produced 10% protein level are shown in comparison to the Fulcaster with a natural 10% protein in Figure 2. The maximum response in the milk-free doughs was obtained with 1 mg.% of bromate; with milk in the formula the requirement was increased to 2 mg.% (or more).

A comparison of the maximum loaf volumes obtained on the four flours is shown in Table I. These results show that addition of shorten-



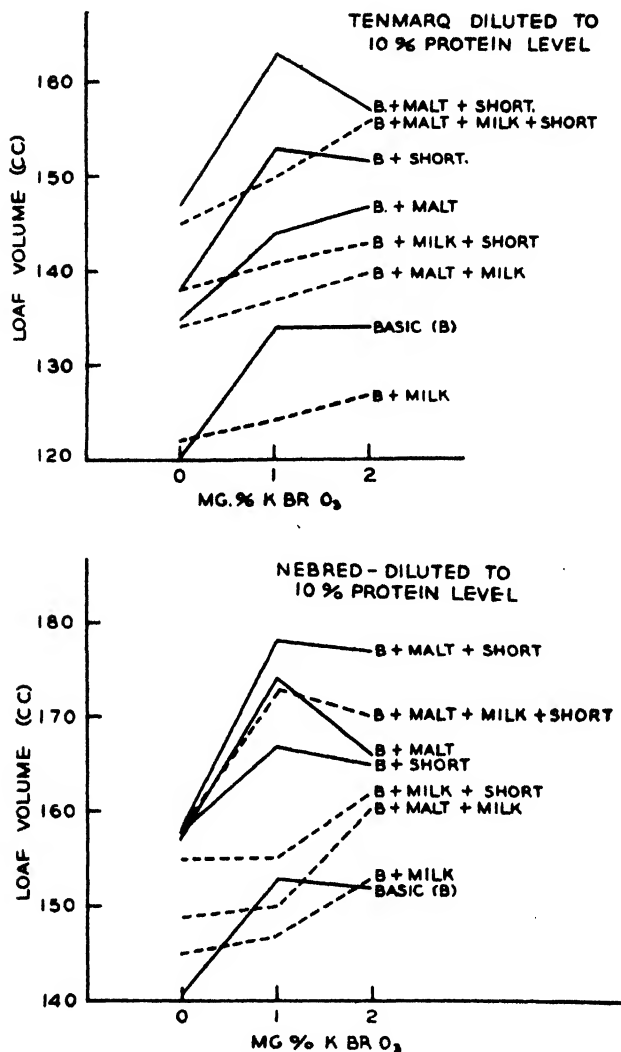
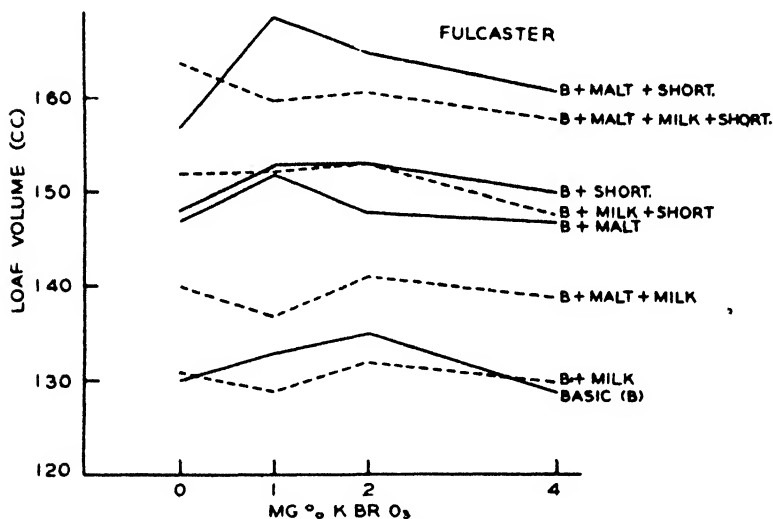
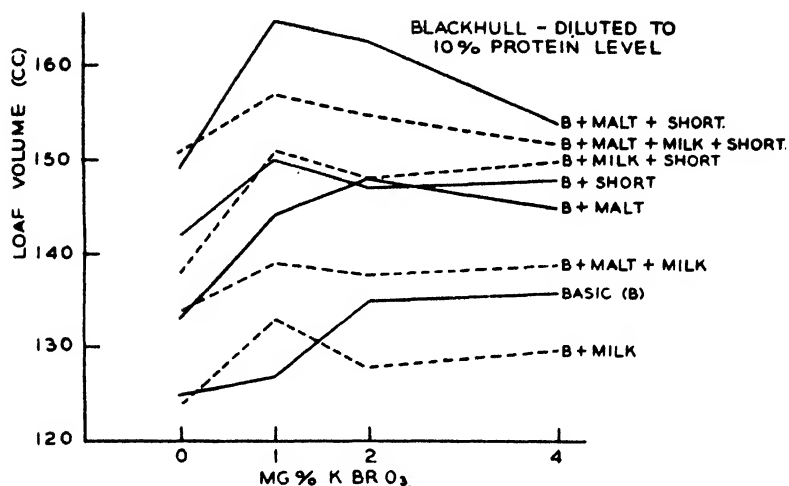


Fig. 2. Comparison of loaf volumes obtained by variations in (Fulcaster 10%)

ing, malt, and milk to the formula has a similar effect on the maximum loaf volume obtained from each of these flours. If the relative value of these flours were to be judged from the loaf volume they would be placed in the same sequence regardless of whether the formula contained malt, milk, or shortening; *i.e.*, the original flours produced loaves which ranged in volume in the order of increasing protein content and the use of supplements did not change this order. This would not necessarily hold true with any other series of flours, since the stimulation obtained by each supplement varies with the flour.

The results obtained by baking the flours at an artificially produced



the baking formula on flours brought to a 10% protein level.  
original protein.)

10% protein level show that it may be possible to use this method to determine quality. The Tenmarq and Blackhull samples gave practically identical loaf volumes with the original Fulcaster, which had a protein content of 10%, while the Nebred sample gave decidedly higher volumes. It would seem from these data that the inclusion of malt, milk, and shortening is not essential for the determination of quality. We are unable to explain the lack of volume stimulation obtained with milk in these bakes, since this definitely does not agree with the results obtained by Larmour, Working, and Ofelt (1939, 1940) or Ofelt and Larmour (1940).

TABLE I

LOAF VOLUMES OBTAINED AT OPTIMUM BROMATE LEVEL WITH VARIOUS FORMULAS

Formula	Ful-caster 9.9% protein	Ten-marq 14.4% protein	Black-hull 15.8% protein	Nebred 16.1% protein	Ten-marq, diluted to 10% protein	Black-hull, diluted to 10% protein	Nebred, diluted to 10% protein
Basic (B)	135	159	178	198	134	136	153
B—shortening	153	175	188	209	153	150	167
B—malt	152	175	188	226	147	148	174
B—milk	132	156	170	198	127	133	153
B—malt—short.	169	179	195	215	163	165	178
B—malt—milk	141	180	175	203	140	139	160
B—milk—short.	153	171	189	192	143	151	162
B—malt—milk—short.	164	175	195	213	156	157	173

### Relation of Quality to Protein Level

The comparative quality of protein at various naturally occurring protein levels may only be determined by studying samples of flour covering a wide range of protein for each of several pure wheat varieties. We were fortunate in obtaining such a variety-protein series of flours through the courtesy of E. G. Bayfield and the Department of Milling Industry of Kansas State College. These samples were baked in duplicate by the same micro technique as described above, using the A. A. C. C. basic formula supplemented with dry milk solids (6%), shortening (3%), malted wheat flour (0.5%), and 4 mg.% of potassium bromate. The loaf volumes obtained are shown graphically in Figure 3. Each curve represents a single variety over a range in protein content. It is notable that there was an exceedingly wide spread in loaf volumes between varieties at any give protein level. The difference increased with increasing protein content. This might indicate that there are greater differences in protein quality at the higher levels.

These flours were diluted to a 10% protein level and rebaked. The formula used differed from that used on the undiluted flours only in that it contained no milk and the bromate was reduced to 1 mg.%. The loaf volumes obtained were plotted against the original protein content of the samples and are illustrated in Figure 4. There was a marked difference between varieties in loaf volumes obtained. The curves for all varieties have a strong tendency to be similar in shape, the shape indicating that the highest-quality protein in any one variety was found in flour which had a naturally occurring protein content of 12% to 13%; perhaps in this particular series the climatic conditions which produced this protein level happen to be the conditions favorable for the production of high baking quality. The protein decreased in quality as the

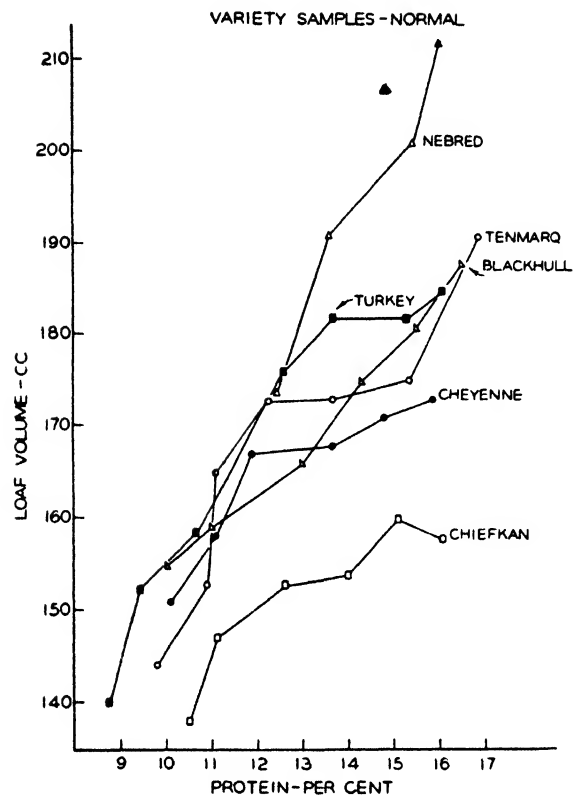


Fig. 3. Loaf volumes obtained on the original variety samples.

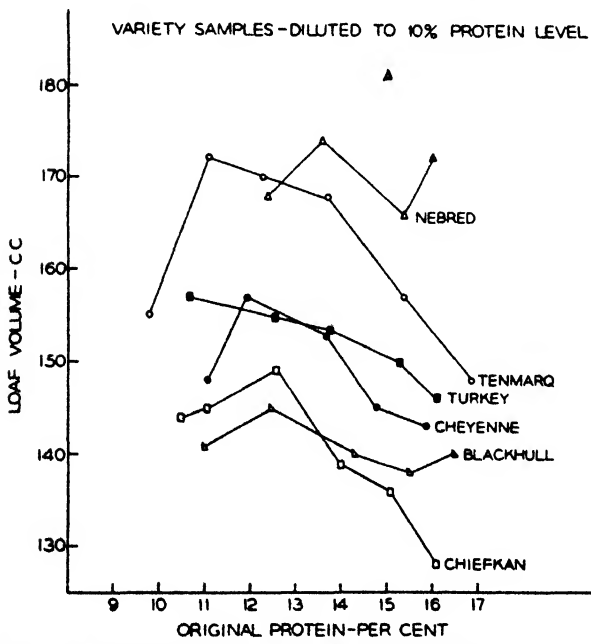


Fig. 4. Loaf volumes obtained on the variety samples reduced to a 10% protein level.

percentage increased above approximately 13%. It should be noted that the high-protein Blackhull flour grown at Lincoln, Nebraska, which was used in the preliminary survey of methods (Figs. 1 and 2), had high protein quality, suggesting that the poor quality indicated in some of the samples may be environmental rather than varietal. Further evidence of this probable effect of environment is given in Figures 8 and 9 by the isolated triangles representing a 15% protein Nebred sample grown at Lincoln, Nebraska. It should be noted that these curves indicate ratings for the varieties that differ from those indicated by the original undiluted flours (Fig. 3).

Figures 5, 6, 7, 8, 9, and 10 show the external appearance of the loaves baked from this variety-protein series of flours. The upper loaves represent the original flours; the lower loaves represent in each

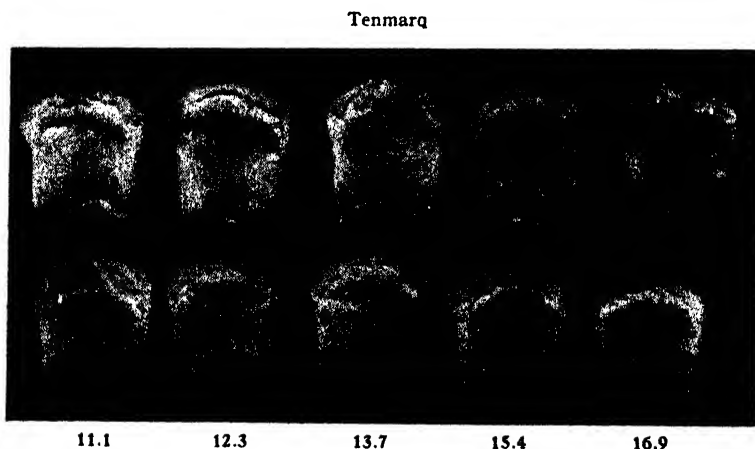


Fig. 5. Comparison of loaves baked from the original Tenmarq flours with loaves baked from the same flours diluted to a 10% protein level. The figures indicate original protein content of the flour.

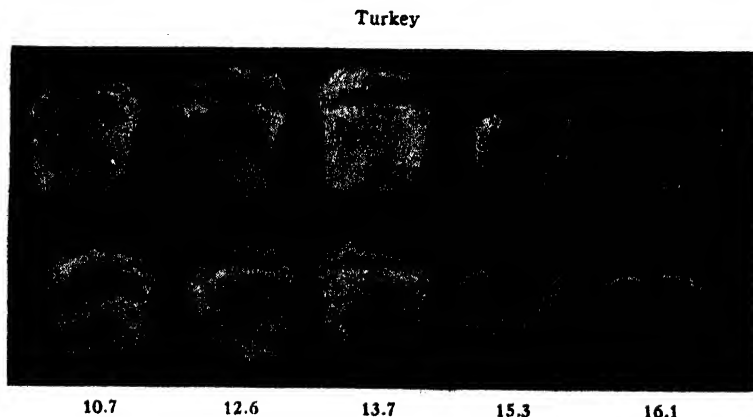


Fig. 6. Comparison of loaves baked from the original Turkey flours with loaves baked from the same flours at an artificial 10% protein level.

Blackhull

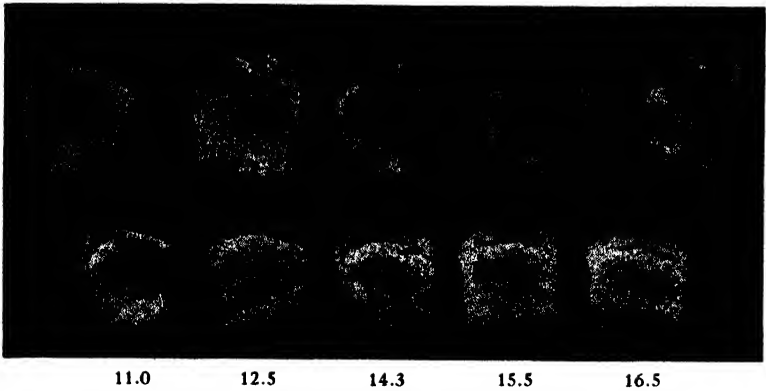


Fig. 7. Comparison of loaves baked from the original Blackhull flours with loaves baked from the same flours at an artificial 10% protein level.

Nebred



Fig. 8. Comparison as above for Nebred.

Cheyenne

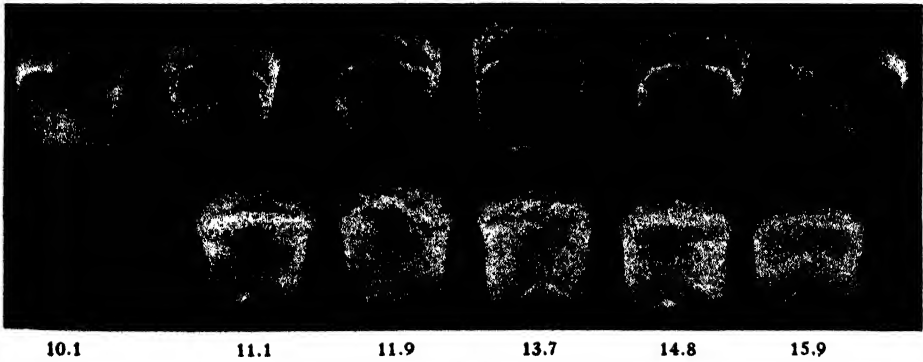


Fig. 9. Comparison as above for Cheyenne.

## Chieffkan

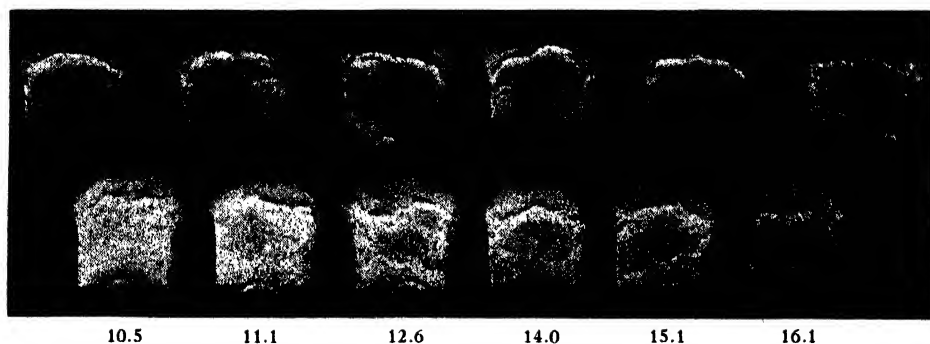


Fig. 10. Comparison as above for Chieffkan.

case the same flour diluted to a 10% protein level. The figure under the loaf indicates the original protein content of the flour. The indication of retrogradation of protein quality with increasing protein content is evident though the variation between varieties in this respect is very marked. Judging from the results obtained on the artificially produced 10% protein flours, Tenmarq (Fig. 5) seemingly had very high protein quality in the intermediate protein range with a rapid decrease with increase in protein content. The quality of the Turkey flours (Fig. 6) was intermediate and quite uniform at all levels. The Blackhull samples (Fig. 7) had rather poor quality over the entire protein range, while the Nebreß samples (Fig. 8) had exceedingly high quality even in the high protein ranges.

### Summary

A method is suggested for comparing the protein quality of flours at a definite protein level.

The applicability to the study of quality of supplements of malt, shortening, and milk to the A. A. C. C. basic formula was investigated. Van Scoyk's micro-baking technique was used. All supplements gave the same comparative evaluations of the flours when the oxidation and mixing times were optimum for the individual flours. The comparative evaluations of flours reduced to a definite protein level differed from the comparative evaluation of the original flours.

A study was made of the comparative quality of the proteins of wheat varieties at various naturally occurring protein levels. The data indicate that there is wide variation in protein quality between varieties and that the protein quality within a variety varies with the naturally occurring protein level. It is suggested that the results obtained by this protein dilution method may be an indication of the blending value of flours.

## Literature Cited

- Aitken, T. R., and Geddes, W. F.  
1934 The behavior of strong flours of widely varying protein content when subjected to normal and severe baking procedures. *Cereal Chem.* 11: 487-504.
- Blish, M. J., and Sandstedt, R. M.  
1935 Definition and measurement of "flour strength" as an inherent property of wheat. *Cereal Chem.* 12: 653-664.
- Larmour, R. K., Working, E. B., and Ofelt, C. W.  
1939 Quality tests on hard red winter wheats. *Cereal Chem.* 16: 733-752.  
1940 Quality tests on soft red winter wheats of Kansas. *Cereal Chem.* 17: 18-29.
- Ofelt, C. W., and Larmour, R. K.  
1940 The effect of milk on the bromate requirements of flours. *Cereal Chem.* 17: 1-18.
- Sandstedt, R. M., Jolitz, C. E., and Blish, M. J.  
1939 Starch in relation to some baking properties of flour. *Cereal Chem.* 16: 780-792.
- Van Scoyk, W. V.  
1939 Micro-baking technique application and results. *Cereal Chem.* 16: 1-12.

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## INCREASE OF VITAMIN B<sub>1</sub> INTAKE BY THE USE OF SPECIAL HIGH VITAMIN B<sub>1</sub> BREAD <sup>1</sup>

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(Received for publication May 13, 1940)

According to the recent report of the Mixed Committee on the Problem of Nutrition of the League of Nations (1938): "There is strong evidence that human dietaries in many parts of the world are more or less deficient in vitamin B<sub>1</sub> although beri-beri is absent." That inadequacies may be commonly encountered in American dietaries is indicated by the reviews of Williams and Spies (1938) and Jolliffe (1938).

Baker, Wright, and Drummond (1937) have traced the progress of flour refining and have indicated the changes which this progress has made in the vitamin B<sub>1</sub> content of the diet. The amount of thiamin in whole wheat is not greatly in excess of that found in several other foods. However, interest is rightly centered in this substance since it has been shown that over 20% of the caloric intake of the American diet is contributed by wheat. For this reason wheat, if not deprived of its vitamin B<sub>1</sub> content by milling procedures, may contribute very significantly to the total intake of the vitamin. However, according to the values given by the investigators cited above, 100 g. of modern white flour contains

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<sup>1</sup> This investigation was aided by a grant from Standard Brands, Inc.

<sup>2</sup> With the technical assistance of Gordon H. Magruder.



only 7% as much vitamin as 100 g. of whole wheat and their surveys indicate that refined white flour accounts for well over 80% of the sales of wheat products for human use.

The need for the addition of vitamin B<sub>1</sub> to staple American foods has recently been stressed by Cowgill (1939). The use of a yeast produced under conditions so that it has a high content of vitamin B<sub>1</sub> has been shown by Schultz, Atkin, and Frey (1939) to restore the thiamin content of white bread so that it is equivalent to whole-wheat bread.

The evaluation of the state of thiamin nutrition by determining the output of the vitamin in the urine has been proposed by Harris and Leong (1936). This has led to the development of several methods for the determination of thiamin (Levine and Marples, 1938). The present report presents studies carried out on seemingly normal young women in which special white bread of high thiamin content was included in the diet in moderate amounts.

### Experimental

Seventeen well nourished young women who expressed a willingness to cooperate served as the subjects in this study. For the most part these were students at Western Reserve University. A preliminary control period was observed during which a 24-hour sample of urine was collected. Hemoglobin estimations and red cell counts were also made during this time. At the end of the control period, which varied from one to three weeks, the subjects were supplied with the special bread and instructed to substitute this bread for the bread ordinarily consumed, but to make no other alterations in their ordinary diet. The subjects ate this bread for approximately five weeks. During this time tabulation of the total food intake for seven consecutive days was made, and from these data the vitamin B<sub>1</sub> intake was calculated as well as the proportion of the total intake of vitamin B<sub>1</sub> supplied by the bread. Hemoglobin estimations and red cell counts were made at fortnightly intervals, and at the end of the bread-eating period two such estimations were made on different days. A second 24-hour sample of urine was collected at the end of this period. The subjects also filled out a questionnaire as to any subjective symptomatic changes that might have been observed during the period of eating the high vitamin B<sub>1</sub> bread.

Estimation of hemoglobin was carried out in all cases by the acid hematin method and by the benzidine method of Bing and Baker (1931). Red cell counts were made as described in standard texts on hematology.

The composition of the diets was calculated from the tables of Waller (1937). The preferred values for the thiamin content of foods given

by Daniel and Munsell (1937) and Williams and Spies (1938) were<sup>1</sup> used in calculating the thiamin intakes.

*Method for estimation of urinary thiamin.*—The urinary thiamin was determined by a modification of the yeast fermentation method of Schultz, Atkin, and Frey (1937, 1938). As suggested by these authors, normal urine appears to contain substances in addition to thiamin which accelerate the rate of yeast fermentation. A technique was used in which the stimulating effect of urine was compared with a similar amount of urine in which the thiamin had been destroyed by treatment with alkaline ferricyanide. Two additional fermentation mixtures were employed which contained urine that had been treated to destroy its original thiamin and to which had been added 2 and 4 gamma of crystalline thiamin.<sup>2</sup> From the stimulatory effect of the known amount of thiamin the concentration of thiamin in the urine was calculated. Schultz and his associates measured the fermentation rate by catching the evolved CO<sub>2</sub> in manometers. We have used this method and have also on the same samples determined the concentration of residual sugar in the fermentation mixtures and thus have evaluated sugar consumption.<sup>4</sup> Values based on calculations of gas production or sugar consumption agree within the limits of the experimental error of the method. It is quite possible of course to carry out analyses for thiamin without the use of manometers if residual sugar estimations are made. These can conveniently be made by saturating a portion of the fermentation mixture with picric acid and then applying the colorimetric sugar method of Myers and Bailey (1916).

Under the conditions employed, the treatment with alkaline ferricyanide always resulted in complete destruction of thiamin if it was added to the urine. Numerous recovery experiments were carried out in which it was possible to ascertain the amount of thiamin that had been added to the urine. In the studies reported below duplicate determinations were carried out on all of the urine specimens.

*Source of bread.*—The bread used in this study was baked twice weekly by a local bakery. It was a plain white bread baked with a high vitamin B<sub>1</sub> yeast. Assays of the thiamin content of the bread were carried out each week by the fermentation method.

## Results

The effect of the ingestion of the high vitamin B<sub>1</sub> bread on the hemoglobin concentration and the red cell counts of the subjects is shown in Table I. Inspection of the individual values indicates that

<sup>2</sup> Kindly supplied by Merck and Co., Inc., Rahway, New Jersey.

<sup>4</sup> This type of procedure was suggested by Dr. J. A. Killian.

TABLE I  
BLOOD FINDINGS IN SUBJECTS EATING HIGH VITAMIN B<sub>1</sub> BREAD

Subject	Average during initial control period		Average at end of bread-eating period	
	Hemoglobin	RBC count	Hemoglobin	RBC count
	<i>g. per 100 cc.</i>	<i>Millions per cu.mm.</i>	<i>g. per 100 cc.</i>	<i>Millions per cu.mm.</i>
1	12.3	4.7	12.2	4.4
2	10.7	4.6	9.8	4.3
3	13.0	4.7	12.6	4.5
4	13.0	4.5	13.0	4.6
5	12.7	4.9	13.4	5.2
6	13.1	4.1	11.4	4.2
7	12.7	4.8	13.7	5.0
8	12.5	4.8	12.8	4.8
9	12.5	4.4	12.3	3.9
10	14.6	4.9	13.7	4.9
11	12.0	4.1	12.9	4.6
12	13.4	4.3	13.0	4.6
13	12.4	4.8	12.5	4.9
14	12.7	4.9	11.8	4.5
15	11.9	4.5	12.0	4.3
16	12.6	4.3	12.0	4.6
17	12.5	4.1	12.6	4.3
Average	12.6	4.6	12.5	4.6

the maximum increase in hemoglobin was 1.0 g. per 100 cc. of blood, whereas the maximum decrease was 1.7 g. The average values for the control period and for the analyses at the end of the bread-eating period are practically identical. There were also no significant changes in the red cell counts.

The effect of the ingestion of high vitamin B<sub>1</sub> white bread on the urinary excretion of thiamin is shown in Table II. The individual values for thiamin excretion vary widely, the lowest value being 39 gamma of thiamin per 24-hour sample and the highest 450 gamma per 24-hour sample. Of the 17 subjects, 14 showed an increase in the urinary thiamin excretion after the period of ingestion of the high vitamin B<sub>1</sub> bread. Of the three subjects who showed a decrease in urinary thiamin two were out of school for two or three days with influenza just prior to collection of the second 24-hour urine specimen. The average figures for the group indicate that the urinary thiamin output was nearly doubled as a result of the ingestion of the vitamin B<sub>1</sub> bread.

A summary of the information obtained from the daily diet records of the subjects appears in Table III. The percentage of the total thiamin intake contributed by the bread is seen to be 34.1%, which is a very considerable proportion.

Of the 17 subjects, 15 reported that they liked the bread and two

TABLE II  
URINARY THIAMIN EXCRETION

Subject	Thiamin excretion during control period before eating vitamin B <sub>1</sub> bread	Thiamin excretion at end of period of ingestion of vitamin B <sub>1</sub> bread
	<i>Gamma per 24 hours</i>	<i>Gamma per 24 hours</i>
1	39	154
2	118	275
3	102	94
4	72	82
5	258	346
6	74	157
7	73	80
8	53	121
9	73	162
10	271	151
11	53	330
12	92	450
13	100	152
14	87	43
15	71	396
16	180	350
17	79	151
Average	106	206

TABLE III  
SUMMARY OF DIETARY RECORDS OF SUBJECTS EATING HIGH VITAMIN B<sub>1</sub> BREAD

Subject	Age	Weight	Protein intake <sup>1</sup>	Fat intake <sup>1</sup>	Carbo- hydrate intake <sup>1</sup>	Caloric intake <sup>1</sup>	Percent of thiamin intake contributed by B <sub>1</sub> bread	
							Thiamin intake <sup>1</sup>	$\gamma$
	<i>yrs.</i>	<i>kg.</i>	<i>g.</i>	<i>g.</i>	<i>g.</i>	<i>cal.</i>	$\gamma$	
1	35	56	64	98	180	1858	913	37.6
2	28	49	97	96	224	2150	1155	30.8
3	20	56	70	78	182	1710	738	39.4
4	25	66	103	164	238	2840	1400	28.2
5	20	57	85	107	219	2179	1134	23.2
6	23	47	62	89	284	2185	911	36.9
7	20	58	70	93	198	1909	1041	37.4
8	20	52	73	87	225	1975	1110	31.5
9	36	70	73	102	249	2206	1251	34.6
10	30	54	78	107	249	2271	1245	37.8
11	22	66	74	74	150	1562	984	32.6
12	24	53	90	115	197	2183	1222	33.3
13	20	60	66	69	231	1807	1005	27.9
14	20	57	59	85	207	1829	933	45.3
15	32	69	77	58	223	1721	1076	37.8
16	33	48	63	86	194	1802	942	34.1
17	22	44	73	87	203	1887	986	31.6
Average	25	57	75	94	215	2004	1062	34.1

<sup>1</sup> Average daily intake.

reported that they did not particularly like any kind of bread but that this was as good as any. The approximate average daily consumption over the whole period was between four and five slices. Four of the subjects reported that they definitely felt better while eating the bread and six reported an increase in appetite. No other changes in subjective symptoms were reported.

### Discussion

For some time there has been a vague suggestion of relationship between vitamin B<sub>1</sub> and secondary anemia. This is indicated by the policy of some physicians to include vitamin B<sub>1</sub> in the treatment of certain secondary anemias. It is also suggested by the policy of some of the pharmaceutical houses who recommend preparations for anemia which are fortified with vitamin B<sub>1</sub>.

Minot (1939) recently stated that more information was needed regarding the relationship between chronic iron deficiency and some factor of the vitamin B complex. Summerfeldt and Ross (1938) found that the addition of vitamin B<sub>1</sub> and iron to a "good" diet of normal children produced increases in hemoglobin concentration of the blood and increases above the expected growth rate. Schlutz, Oldham, and Morse (1938) have suggested that increases in vitamin B<sub>1</sub> intake result in a decrease in iron retention in the normal infant.

The exact status of our subjects was somewhat questionable. In light of present-day hematological standards, the hemoglobin concentrations were definitely below the figure that is normally given for such an age group. However, for three successive years surveys in this laboratory of the hemoglobin levels in similar groups have given almost identical results. Furthermore if this is a true anemia it does not respond to administration of iron salts since a large group of such students did not show hemoglobin increases when fairly large doses of iron were given (unpublished observations from this laboratory). In the present study the ingestion of increased amounts of vitamin B<sub>1</sub> as supplied by the bread did not appear to have any effect on the hemoglobin concentration or the red cell counts in these subjects. If a true mild anemia existed it must have been due to other factors.

Since the original work of Harris and Leong (1936) numerous studies of thiamin excretion have been made. Jolliffe, Goodhart, Gennis, and Cline (1939) noted that definite clinical symptoms of deficiency developed in four of five male subjects when the excretion level dropped below 100 gamma per 24 hours. Melnick, Field, and Robinson (1939) have found a definite difference in the excretion levels of male and female subjects. These investigators found that a group of nine women

consuming their ordinary diet excreted 93 gamma of thiamin per 24 hours. The calculated dietary intake of thiamin was 710 gamma per 24 hours. In the subjects of the present study the daily thiamin excretion during the control period was 106 gamma and from the dietary records it might be assumed that they receive approximately 750 gamma of thiamin per day. The agreement between these two series is quite striking. Melnick, Field, and Robinson suggest that a thiamin excretion of 60 to 70 gamma per 24 hours for an adult female represents a borderline condition with respect to thiamin intake. In their series and our own the excretion was not greatly above this level nor was the amount of thiamin in the diet greatly in excess of the minimum requirement suggested by Cowgill (1938). In the series of Melnick, Field, and Robinson (1939) the women were wives of hospital staff members and in the present series the subjects were young college women. In our series we feel that in no case was the ordinary diet greatly restricted by the economic status of the subject.

With the inclusion of the vitamin B<sub>1</sub> bread in the ordinary diet a very definite increase in the average thiamin excretion in the urine was noted at the end of the bread eating period. Approximately 20% of the thiamin intake appeared in the urine. This increase in percentage of excretion might be expected since the increased vitamin intake continued for a period of 5 weeks during which time there was an opportunity for any depleted reserves to be restored. Furthermore the possibility of partial destruction of thiamin in the gastro-intestinal tract which has been suggested as occurring when large doses are given during the fasting state (Melnick *et al.* 1939) is minimized since the extra thiamin was obtained from the food eaten with the three daily meals. There can be little question that if thiamin excretion reflects the level of thiamin nutrition (Melnick *et al.*, 1939; Jolliffe *et al.*, 1939) the subjects in this study were in a much better state of thiamin nutrition as a result of substituting the special vitamin B<sub>1</sub> white bread in place of that ordinarily included in the diet.

It is realized that analysis of diet records is subject to a considerable error as far as the amounts of foods are concerned. Furthermore a marked variation occurs in the composition of different samples of the same food. The dietaries of this group of subjects during ingestion of the special bread as indicated by Table III included adequate amounts of vitamin B<sub>1</sub>. The last column of Table III indicates the amount of thiamin contributed by the high vitamin B<sub>1</sub> bread, and it is to be seen that the thiamin content of the bread makes up over 34% of the total thiamin intake. In these diets the bread contributed approximately 16% of the caloric intake. It is well recognized that low-income groups have the most limited thiamin intake. In these same diets bread contributes

a larger percentage of the total caloric intake. In such low-income groups the use of vitamin B<sub>1</sub> bread might reasonably be expected to cause more marked improvement in the thiamin intake than noted in the present study.

In regard to the subjective symptoms very little can be said since this merely represents the reports of the subjects. However the possibility that the changes in general well being and in appetite reported by 25% of the subjects were real rather than psychological cannot be denied.

### Summary

White bread baked with a new high vitamin B<sub>1</sub> yeast was ingested by 17 young college women in place of the bread ordinarily included in the diet. The somewhat subnormal blood hemoglobin concentrations of these subjects did not show any alteration during this period. The urinary thiamin excretion before the period of eating the special bread averaged 106 gamma per 24 hours, whereas at the end of the five-week period the excretion of thiamin averaged 206 gamma per 24 hours. If the urinary excretion reflects the state of thiamin nutrition, this indicates an improved state of thiamin nutrition in these subjects. Dietary records obtained from the subjects indicated that the special bread contributed 34% of the total thiamin intake.

### Literature Cited

- Baker, A. Z., Wright, M. D., and Drummond, J. C.  
1937 The nutritive value of bread. *J. Soc. Chem. Ind.* **56**: 191-194.
- Bing, F. C., and Baker, R. W.  
1931 The determination of hemoglobin in minute amounts of blood by Wu's method. *J. Biol. Chem.* **92**: 589-600.
- Cowgill, G. R.  
1938 Human requirements for vitamin B<sub>1</sub>. *J. Am. Med. Assoc.* **111**: 1009-1016.  
1939 The need for the addition of vitamin B<sub>1</sub> to staple American foods. *J. Am. Med. Assoc.* **113**: 2146-2151.
- Daniel, E. P., and Munsell, H. E.  
1937 Vitamin content of foods. U. S. Dept. Agri. Misc. Pub. No. 275.
- Harris, L. J., and Leong, P. C.  
1936 Excretion of vitamin B<sub>1</sub> in human urine and its dependence on dietary intake. *Lancet* **1**: 886-894.
- Jolliffe, N.  
1938 A clinical evaluation of the adequacy of vitamin B<sub>1</sub> in the American diet. *New International Clinics* **IV**, Series **48**: 47-67.
- , Goodhart, R., Gennis, J., and Cline, J. K.  
1939 The experimental production of vitamin B<sub>1</sub> deficiency in normal subjects. The dependence of the urinary excretion of thiamin on the dietary intake of vitamin B<sub>1</sub>. *Am. J. Med. Sci.* **196**: 198-211.
- League of Nations  
1937 Final report of the mixed committee of the League of Nations on the relation of nutrition to health, agriculture, and economic policy. Columbia University Press, New York. No. **A13**, p. 62.
- Levine, S. Z., and Marples, E.  
1938 The status of the color tests for vitamin B<sub>1</sub>. *Nutrition: The newer diagnostic methods.* Milbank Memorial Fund, New York. 91-101.

- Melnick, D., Field, H., and Robinson, W. D.  
1939 A quantitative chemical study of the urinary excretion of thiamin by normal individuals. *J. Nutrition* **18**: 593-610.
- Minot, G. R.  
1939 *Anemias of nutritional deficiency. A symposium on the blood and blood-forming organs.* University of Wisconsin Press, Madison, Wisconsin.
- Myers, V. C., and Bailey, C. V.  
1916 The Lewis and Benedict method for the estimation of blood sugar with some observations obtained in disease. *J. Biol. Chem.* **24**: 147-161.
- Schlutz, F. W., Oldham, H., and Morse, M.  
1938 Effect of vitamin B<sub>1</sub> on the iron retention of a normal infant. *Am. J. Dis. Child.* **56**: 735-743.
- Schultz, A. S., Atkin, L., and Frey, C. N.  
1937 A fermentation test for vitamin B<sub>1</sub>. *J. Am. Chem. Soc.* **59**: 2457-2460.  
1938 The specificity of the fermentation test for vitamin B<sub>1</sub>. *J. Am. Chem. Soc.* **60**: 3084-3085.  
1939 The vitamin B<sub>1</sub> contents of wheat, flour and bread. *Cereal Chem.* **16**: 643-648.
- Summerfeldt, P., and Ross, J. R.  
1938 Value of increased supply of vitamin B<sub>1</sub> and iron in the diet of children. *Am. J. Dis. Child.* **56**: 985-988.
- Waller, D. S.  
1937 *Nutritive value of foods.* George Wahr, Publisher, Ann Arbor, Mich.
- Williams, R. R., and Spies, T. D.  
1938 *Vitamin B<sub>1</sub> (thiamin) and its use in medicine.* The Macmillan Company, New York.

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## THE THIAMIN CONTENT OF WHOLE-WHEAT AND CLEAR FLOURS

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The fermentation method for the determination of thiamin as developed by Schultz, Atkin, and Frey (1937, 1938) has given the baking technologist a useful tool for the study of the vitamin B<sub>1</sub> content of his raw materials. Compared to feeding tests, the fermentation method is rapid and inexpensive, and therefore of great value in a study of this kind. The data presented in this report (Tables I-IV) are taken from continuous routine assays, and many of the tests were made on flours actually used in production. Before a complete picture of the vitamin B<sub>1</sub> content of flours used in this country can be drawn, statistical studies from many sources as well as different methods of assay must be evaluated, and for this reason these tests should be of interest and serve a useful purpose.

Williams and Spies (1938) have summarized the literature and report variations of from 4.7 to 10.2 gamma of thiamin per gram in whole-wheat flours, and with the preferred average from 4 to 5 gamma



TABLE I  
THIAMIN CONTENT OF SPECIAL WHOLE-WHEAT FLOURS  
(Data calculated to the 10% moisture basis)

Source	Ash	Thiamin	Source	Ash	Thiamin
	%	<i>Gamma/gram</i>		%	<i>Gamma/gram</i>
New York State	1.74	6.8	Pacific Coast	1.56	7.0
New York State	1.48	5.2	Durum (N.W.)	1.69	6.6
New York State	1.78	7.0	Kansas	1.71	7.0
Oklahoma	1.66	6.6	Kansas	1.88	7.6
Texas	1.60	7.7	Kansas (all Chiefkan)	1.71	7.2
Texas	1.75	8.0	Kansas (all Tenmarq)	1.51	5.0
Virginia	1.77	6.7	Kansas (all Turkey)	1.82	6.7
Maryland	1.79	5.2	Northwestern	1.79	7.9
Ohio	1.83	6.4	Northwestern	1.65	8.0
Pacific Coast	1.61	7.8	Northwestern	1.81	6.5
Pacific Coast	1.53	7.5	Southwestern	1.80	7.8
Pacific Coast	1.35	6.0	Southwestern	1.46	5.9

Average: 6.8 gamma per gram

TABLE II  
THIAMIN CONTENT OF COMMERCIAL SHIPMENTS OF WHOLE-WHEAT FLOUR  
(Data calculated to 10% moisture basis)

Ash	Thiamin	Ash	Thiamin
%	<i>Gamma/gram</i>	%	<i>Gamma/gram</i>
1.75	5.3	1.58	6.6
1.75	8.7	1.54	6.9
1.81	7.9	1.77	6.5
1.78	7.6	1.76	6.7
1.77	8.2	1.51	6.5
1.81	7.1	1.57	6.6
1.76	6.7	1.55	6.2
1.84	8.0	1.62	6.6
1.56	6.7	1.74	6.7
1.77	7.8	2.05	6.4
1.55	7.7	1.58	5.7

Average: 6.9 gamma per gram

TABLE III  
VARIATION OF THIAMIN CONTENT OF WHOLE-WHEAT BREAD WITH THE  
THIAMIN CONTENT OF THE FLOUR USED

Flour (10% moisture basis)		Bread (38% moisture)	
<i>Gamma/gram</i>	<i>Int. units/lb.</i>	<i>Int. units/lb.</i>	<i>Decrease, %</i>
5.3	800	460	42.5
7.8	1182	650	45.0
6.5	984	550	44.0

TABLE IV  
THIAMIN CONTENT OF CLEAR FLOURS  
(Data calculated to the 13% moisture basis)

Ash	Thiamin
%	<i>Gamma/gram</i>
0.534	2.7
0.540	3.4
0.563	2.8
0.574	2.0
0.615	2.3
0.684	3.1
0.698	2.6
0.706	3.0
0.710	2.5
0.710	3.1
0.712	2.8
0.723	2.6
0.727	4.3
0.753	4.4
0.754	3.9
0.760	3.6
0.764	4.1
0.808	4.7

per gram. These figures are taken from feeding tests and from the bradycardia method of assay, and a considerable number of them represent the thiamin content of flours available in England.

The decrease in vitamin B<sub>1</sub> content from the flour to the bread represents a combination of factors: first, the dilution due to the higher moisture content of the bread as compared to the flour; secondly, the addition of nonvitamin-containing ingredients such as shortening, sugar, and salt; and thirdly, a slight baking loss.

Copping and Roscoe (1937) summarized the literature then available on clear flours. The results reported indicated that the lower the grade of the flour, the higher the vitamin content. Schultz, Atkin, and Frey (1939) found clear flours to contain 2.7 and 1.8 gamma of thiamin per gram, and a second-grade clear to contain 5.1 gamma per gram.

In Figure 1 some values for cake, standard-patent, and straight-patent flours were included in order to illustrate further the variation of the thiamin content with the ash of the flour. It is interesting to note that whatever the degree of extraction or the source of the flour, the thiamin content bears a quite definite relationship to the ash.

### Summary

As determined by the fermentation method the average thiamin content of 46 samples of whole-wheat flour was 6.85 gamma per gram on

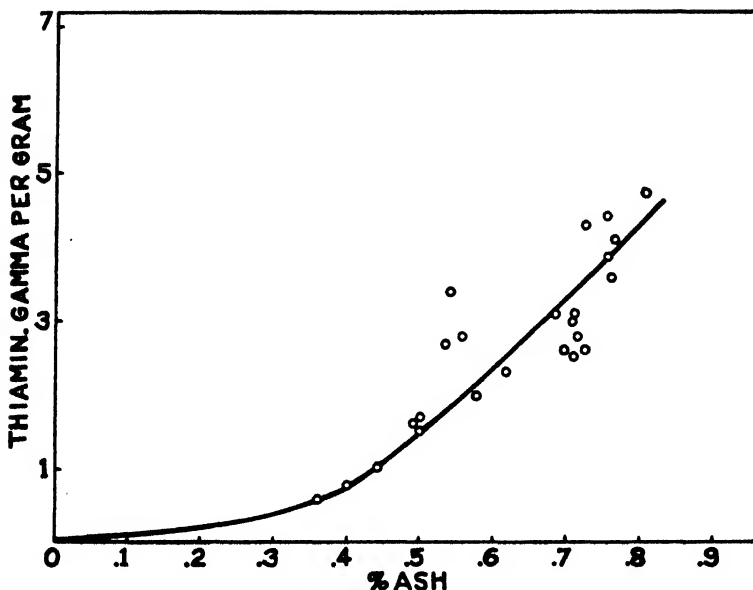


Fig. 1. The relationship between thiamin content and the ash of flour.

the 10% moisture basis. There was no correlation with ash content and no special trend toward high or low vitamin contents in various parts of the wheat-producing areas except that the white or softer type of wheat tended to be somewhat lower than the average. Most of the samples tested were no doubt blends of wheat varieties and hence no correlation with varieties could be suggested.

The thiamin content of clear flours had a definite relationship to the ash content and varied from 2.0 to 4.7 gamma per gram.

#### Literature Cited

- Copping, A. M., and Roscoe, M. H.  
1937 The water-soluble B-vitamins in yeast, flour and bread. *Biochem. J.* 31: 1879-1902.
- Schultz, A. S., Atkin, L., and Frey, C. N.  
1937 A fermentation test for vitamin B<sub>1</sub>. II. *J. Am. Chem. Soc.* 59: 2457-2460.  
1938 Influence of nicotinic acid on the fermentation method for vitamin B<sub>1</sub>. *J. Am. Chem. Soc.* 60: 1514.  
1939 The vitamin B<sub>1</sub> content of wheat, flour and bread. *Cereal Chem.* 16: 643-648.
- Williams, R. R., and Spies, T. D.  
1938 Vitamin B<sub>1</sub> and its use in medicine. The Macmillan Co.

# THE LOSS OF THIAMIN IN BREAD ON BAKING AND TOASTING

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The feeding tests which have been made on bread and bread ingredients in order to obtain the vitamin B<sub>1</sub> content indicate that the baking loss is small. For example, Copping and Roscoe (1937) pointed out that the similarity of the vitamin contents of the breads and the flours from which they were made suggested no appreciable destruction of the vitamin during baking. Williams and Spies (1938) reported that the vitamin content of the bread on the air-dried basis was similar to that of the flour from which the bread was made. Aughey and Daniel (1940) found that there was a baking loss of 15% in whole-wheat bread. These results suggest that the baking loss is probably of a degree which approaches the limit of accuracy of a feeding test.

The fermentation test (Schultz, Atkin, and Frey, 1937, 1938) gives results which indicate that the baking losses vary from 5% to 9% of the total vitamin originally present. Objections may be made to the use of the fermentation test in this type of study because of the possible yeast-stimulating power of break-down products of the thiamin. If such were entirely true, it would be difficult to show any baking loss by this test. Any such possible error, as will be seen, cannot exceed the allowable error of duplicate feeding tests, and the fermentation test therefore gives a satisfactory method for studying the variation in thiamin content of the different portions of the slice, a study which would be difficult with feeding tests.

In order to determine the thiamin content of different portions of the slice, and thereby measure the baking loss, slices were divided as follows: (1) the caramelized part of the crust, (2) one-half inch of the slice immediately under the crust, and (3) the interior of the slice (one square inch). These various portions were air-dried, ground, and the thiamin determined. The results in all cases were calculated to the dry basis.

The assumption was made that no destruction of thiamin took place in the immediate center of the loaf, and therefore the baking loss was taken as the percentage difference between the thiamin content of the interior of the slice and the average of the entire slice. The average baking loss of the three types of bread shown in Table I was 7%.

No doubt the end slices would lose proportionally more than the

TABLE I

## THIAMIN CONTENTS OF VARIOUS PORTIONS OF THE SLICE

(Thiamin in gamma per gram of bread calculated to the dry basis)

Type of bread	Crust	Next to crust	Interior	Entire slice	Baking loss
Regular white	1.0	1.2	1.2	1.1	8%
High B <sub>1</sub> white	6.9	8.7	10.8	10.2	5%
Whole wheat	5.3	6.7	7.5	6.8	9%

other slices of the loaf because of the greater surface exposed to the heat of the oven, but figured on the entire loaf, the thiamin content of a slice gives a reliable value for the thiamin content of the entire loaf. During the preparation of a number of loaves for feeding tests, the fermentation test indicated no measurable difference in thiamin content between an average slice and the entire loaf, and apparently any effect of the end slices is lost because of the small percentage of the entire loaf represented by the end slices.

Since a rather large portion of the bread consumed in this country is in the form of toast, the stability of thiamin during toasting is of nutritional importance. In order to study the loss of vitamin during toasting, a number of slices from the same loaf were taken and light, medium, and heavy toast prepared. It was difficult of course to obtain exactly the same degree of toasting for the different breads studied, but it is believed, however, that the heavy toast represented considerably more toasting than the average person would prefer. After toasting, the slices, including an untoasted slice serving as a control, were air-dried, ground, and tested for vitamin B<sub>1</sub> by the fermentation method. Results are shown in Table II, and for Melba toast in Table III.

TABLE II

## LOSS OF THIAMIN ON TOASTING

(Thiamin in gamma per gram calculated to the dry basis)

Type of bread	Untoasted	Light toast	Medium toast	Heavy toast	Percentage loss		
					Light	Medium	Heavy
Regular white	1.0	1.0	1.0	1.0	0%	0%	0%
High B <sub>1</sub> white	4.9	4.7	4.2	3.7	4%	10%	24%
High B <sub>1</sub> white	12.3	10.8	10.2	9.7	12%	17%	21%
Whole wheat	6.7	6.7	6.5	5.9	0%	3%	12%

TABLE III  
THE LOSS OF THIAMIN IN THE PREPARATION OF MELBA TOAST  
(Thiamin in gamma per gram calculated to the dry basis)

Type of bread	Untoasted	Melba toast	Percentage loss
Regular white	1.1	1.0	9%
High B <sub>1</sub> white	10.2	7.5	26%

### Summary

The baking losses of thiamin in three types of bread vary from 5% to 9% as measured by the fermentation test.

The toasting losses of thiamin (medium toasting) vary from 0% to 17%, depending on the type of bread. The process of making Melba toast is more destructive of thiamin than ordinary toasting.

### Literature Cited

- Aughey, E., and Daniel, E. P.  
1940 Effect of cooking on the thiamine content of foods. *J. Nutrition* **19**: 285-296.
- Copping, A. M., and Roscoe, M. H.  
1937 The water-soluble B-vitamins in yeast flour and bread. *Biochem. J.* **31**: 1879-1902.
- Schultz, A. S., Atkin, L., and Frey, C. N.  
1937 A fermentation test for vitamin B<sub>1</sub>. II. *J. Am. Chem. Soc.* **59**: 2457-2460.
- 1938 Influence of nicotinic acid on the fermentation method for vitamin B<sub>1</sub>. *J. Am. Chem. Soc.* **60**: 1514.
- Williams, R. R., and Spies, T. D.  
1938 Vitamin B<sub>1</sub> and its use in medicine. The Macmillan Co.

## SOME PHYSICAL AND CHEMICAL CHANGES OCCURRING IN HARD RED SPRING WHEAT GLUTEN DURING DOUGH FERMENTATION

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The effects of dough fermentation upon the properties of wheat gluten have received substantial attention in cereal chemistry literature. Since gluten forms the framework of the dough, holding in its interstices the other substances such as starch, sugar, and yeast cells which are present, any changes induced in the properties of gluten during fermentation are of direct practical importance.

Proteolysis is probably one of the most important factors concerned in fermentation. Too great a proteolytic activity will cause the dough to become distinctly slack, and may even render it entirely unfit for bread-making purposes. Too little activity, on the other hand, may cause a dough to be harsh and "bucky." The hydrogen-ion concentration of the reaction medium has a profound influence upon enzymatic activity and protein behavior. The effects of fermentation and of proteolytic enzymes upon dough and gluten characteristics have been extensively studied, with emphasis upon viscosity changes as indicative of proteolytic effects.

Henderson, Fenn and Cohn (1919) believed that a dough with low viscosity would produce a better loaf of bread than one with high viscosity, and thought that the principal advantage in bringing a dough to a pH of 5 before baking was the minimum viscosity obtained at that hydrogen-ion concentration. Sharp and Gortner (1924) postulated that viscosity of a water suspension prepared from a dough, with electrolytes removed, increased to a maximum as fermentation progressed. In a similar way the viscosity of such a suspension treated with lactic acid markedly increased as fermentation proceeded. Malt extract greatly decreased these effects, presumably as a result of its proteolytic action upon the gluten. It therefore appears from this work that proteolysis decreased viscosity, as would be expected, but that fermentation with the production of acid had an opposite effect. Olsen and Bailey (1925) did not agree with the findings of Sharp and Gortner, but thought that the increased hydrogen-ion concentration of the flour suspension undergoing fermentation reduced viscosity. These workers did not find much proteolysis caused by yeast in doughs.

Sharp and Elmer (1924) reported that the proteolytic enzymes present in wheat flour were capable of attacking and digesting flour protein significantly during a digestion period of two to three weeks. The method of estimating protein changes was by autolyzing flour suspensions at 35° C. and then determining the proteins soluble in  $K_2SO_4$ , ethyl alcohol, etc.

Bailey and Johnson (1924) estimated the loss of carbon dioxide from fermenting doughs by two methods, one involving measurements of expansion of the dough plus loss of  $CO_2$ , and the other the expansion of the dough alone. The loss was found to increase rapidly after a lapse of 100 to 180 minutes, and they judged this point to be an indication of the optimum fermentation period. Sharp and Schreiner (1926) used the Sharp and Elmer method of protein analysis after autolysis but did not find marked proteolysis in dough fermentation. The plasticity constants represented by the consistency and the yield value of glutenin

suspensions after treatment with lactic acid did increase, however, as yeast fermentation progressed.

Brownlee and Bailey (1930) also investigated the proteolysis of bread doughs. Determinations of nonprotein nitrogen by protein precipitation with stannous chloride indicated a relatively unimportant cleavage of gluten protein as fermentation progressed, even in dough to which malt preparations had been added. There was no evidence to show that proteolytic enzymes and the by-products of fermentation, such as acid, alcohol, etc., effected gluten degradation during the time range of usual bread-dough fermentation. Viscosimetric technique, however, did show evidence of changes taking place in fermenting doughs, probably due to differences in imbibitional properties of the flour gluten.

Smith (1925) found that flours of high viscosity were of excellent baking quality, while flours of low viscosity ranged from excellent to poor in baking quality. Smith accordingly postulated that in the majority of instances viscosity was not a measure of baking quality. The viscosities were determined upon leached and unleached flour-water suspensions with a Sheely pipette.

Bohn and Bailey (1936) constructed a "stress meter" and measured the stress-strain relationships of fermenting doughs. A significant reduction in the stress readings occurred during fermentation, which suggested to the authors that progressive fermentation "mellows" rather than "develops" wheat-flour dough. It was also found that high stress readings were a good indication of the ability of a dough to withstand prolonged mixing. Later (1937) these workers found that small amounts of papain did not affect stress readings. Larger additions, however, significantly decreased stress readings. They explained these effects by the hypothesis that small increments of the enzyme act as a flour coagulant, whereas larger amounts are liquefying.

The action of various inhibitory substances upon proteases has been extensively discussed in the literature and will therefore not be reviewed in the present paper. The work of Read and Haas, Jorgensen, Flohil, Harris and Johnson (see "Literature Cited") has called attention to this phase of cereal investigations, with adequate literature references. These researches (especially in the instance of Harris and Johnson) have mainly dealt with the effect of oxidizing agents, such as potassium bromate, upon proteases either native to the flour or added in the form of yeast, papain, pepsin, etc. Harris and Johnson (1940) studied the effects of these enzymes and  $\text{KBrO}_3$  upon viscosity and other properties of spring wheat gluten dispersions. It was found that papain and pepsin have similar effects upon the rate at which glutens dispersed in 10% sodium salicylate, and that bromate altered this rate. The quantity of protein precipitated from the gluten dispersions by  $\text{MgSO}_4$  was mark-



edly reduced by papain, but not by pepsin. The mode of procedure in this study was to add the enzyme and bromate in appropriate quantities to the other dough ingredients, mix for three minutes in the Hobart-Swanson to thoroughly incorporate the ingredients in the dough, and immediately wash the gluten from the dough with a standardized stream of 0.1% sodium phosphate solution of pH 6.8. No time was allowed for enzymic action other than during the mixing period.

In view of the results obtained when the substrate was exposed to the action of the enzyme for a maximum of less than five minutes, and with the work of other investigators upon fermenting doughs in mind, it seemed desirable to extend the study to a determination of enzymic effects upon these properties of wheat gluten during normal dough fermentation. The techniques already described were accordingly applied to doughs which were fermented for varying periods of time.

### Experimental Material and Methods

A commercially milled hard red spring wheat straight flour was used for this investigation. This flour was not bleached nor had any diastatic agent been added. The protein content of the flour was 13.2% and the ash 0.44% on a 13.5% moisture basis.

The doughs from which the glutens were washed were made up in the manner normally used in this laboratory, using the standard basic formula with 5% of sucrose added to furnish sufficient fermentable material for the yeast during the course of the fermentation. It was decided to ferment the doughs over a period of five hours to cover thoroughly the range of customary experimental baking, and to take samples for washing gluten at hourly intervals starting with the first sample immediately after mixing, as had been done in the former study. Each dough would thus furnish six samples of gluten for investigation, and the effect of fermentation upon gluten properties as related to formula could be followed. The doughs were fermented at 30° C. and 85% relative humidity. Punches were made at hourly intervals.

In addition to using the viscosity and fractionation procedures outlined by the authors in previous publications, determinations of hydrogen-ion concentration and oxidation-reduction potentials were made upon the doughs at hourly intervals, using a Beckmann pH meter with glass and platinum electrodes. Because a test of the physical properties of the gluten as influenced by fermentation seemed desirable, a series of resistance tests were made upon the freshly washed gluten by means of a "tenderness" tester. This apparatus was fully described by Binnington, Johansson, and Geddes (1939) and used by them as well as by Harris and Knowles (1940) in determining the tenderness of cooked

macaroni. The possibility of using the tenderness test to study the resistance of doughs and glutes to pressure was privately suggested by Binnington and Geddes inasmuch as the equipment commonly used for determining gluten qualities depends upon stretching and pulling effects. On the other hand the experimental baker largely forms his opinion of gluten quality during the fermentation period by the "feel" of the dough between his hands when punching and working the dough and the results yielded by the apparatus described should correspond in some degree at least to the baker's judgment. A plunger 50 mm. in diameter was used in these experiments.

A modification was introduced in the method of dispersing the pieces of gluten washed from the doughs in sodium salicylate. In former instances this dispersion had been accomplished by shaking the dispersion flasks by hand, at approximately five-minute intervals, care being taken to prevent foaming. No doubt variations in the degree of shaking would occur between operators and between different shaking periods. It was also impossible to follow the shaking schedule throughout the 24 hours, and this factor no doubt introduced small errors in the final results. A further undesirable source of variation was that possible surface denaturation might occur if the shaking were done too vigorously. In view of these considerations a mechanical rotary shaker was constructed of aluminum mounted upon a wooden base. This equipment, a picture of which is shown in Figure 1, is capable of holding 20 Erlenmeyer flasks of 200-cc. capacity at one time. It has a speed of approximately 14 rpm. and is actuated by a  $\frac{1}{4}$  hp. electric motor. This machine functioned very satisfactorily, no trace of foaming being observed in the dispersions during the process of disintegration, while the rate of revolution was sufficient to insure thorough stirring of the flask contents. A further factor in favor of machine shaking was that there would be little opportunity for mechanical disintegration to affect the gluten dispersion rate because of the gentle agitation of the flask contents.

It was decided to study the effect of additions of 0.002% and 0.004% papain to the standard basic formula. An addition of 0.001%  $\text{KBrO}_3$  was also made to the standard basic formula, and increments of 0.001% and 0.004%  $\text{KBrO}_3$  were superimposed upon the 0.004% papain-treated doughs.

### Discussion of Results

As the data are conveniently suited to graphic presentations, no tables of results are presented in this paper, and the discussion will be confined to the figures. The comparative rates of dispersion, as registered in centipoises  $\times 10^3$  and determined at intervals of four hours, will be discussed first.

Examining the data obtained with the standard basic method, as graphically presented in Figure 2, it is evident that the length of time the dough was fermented had a decided effect upon the viscosity-time relationships. The initial effect of fermentation appeared to slightly increase the rate of dispersion in sodium salicylate, while further fermentation decreased it. This effect reached a maximum after three hours. The difference between the second and third hours is very striking, and may be connected with coagulation of the protein by the proteolytic enzymes present in the dough. After the third hour the rate of dispersion increases again, rising approximately half of the distance to the zero hour curve. This rise is seemingly related to further liquefying action of the proteases as well as to an increase in hydrogen ion concentration in the fermenting dough.

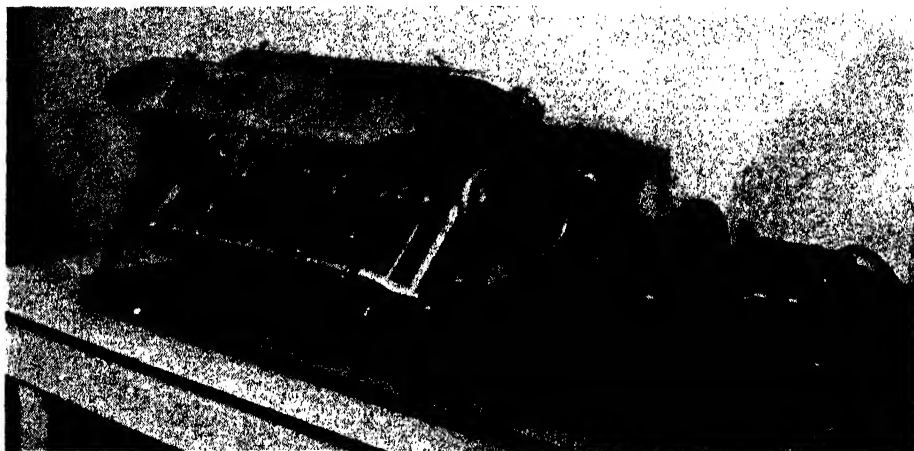


Fig. 1. Motor-actuated rotary shaker used for gently agitating gluten dispersions in 10% sodium salicylate solution during the period of dispersion. Speed-reducing pulleys shown at right. Frame holding flasks is constructed from aluminum and is mounted on steel supports. Rods for fastening flasks in shaker are of brass and are held in correct position by brass thumb screws which can be seen projecting from the sides of the flask carriers. The flasks (200-cc. Erlenmeyer in the photograph) rest in a suitable hollowed-out seat lined with felt. Capacity of shaker, 20 flasks. Speed of shaker, 14 rpm.

Turning next to Figure 3, which represents the data obtained in a similar way with the standard basic formula plus 0.001%  $\text{KBrO}_3$ , the effects of fermentation noticeable in Figure 2 have been increased by the bromate. The zero and first-hour dough curves are very similar to those in Figure 2, but the following four doughs yielded glutens having practically the same dispersion rate. This rate shows a very marked change after the eighth hour of dispersion and probably represents the combined effects of an initial coagulative action by the protease and an inhibitory effect of the bromate, the latter substance acting to prevent further liquefying activity by the proteases.

In Figure 4 the results obtained when 0.002% papain was added to the dough mix are shown. There are no apparent significant differences among the curves representing the glutens washed from doughs fermented for different periods. In comparing this set of results with the preceding figures it is evident at once that the glutens all dispersed

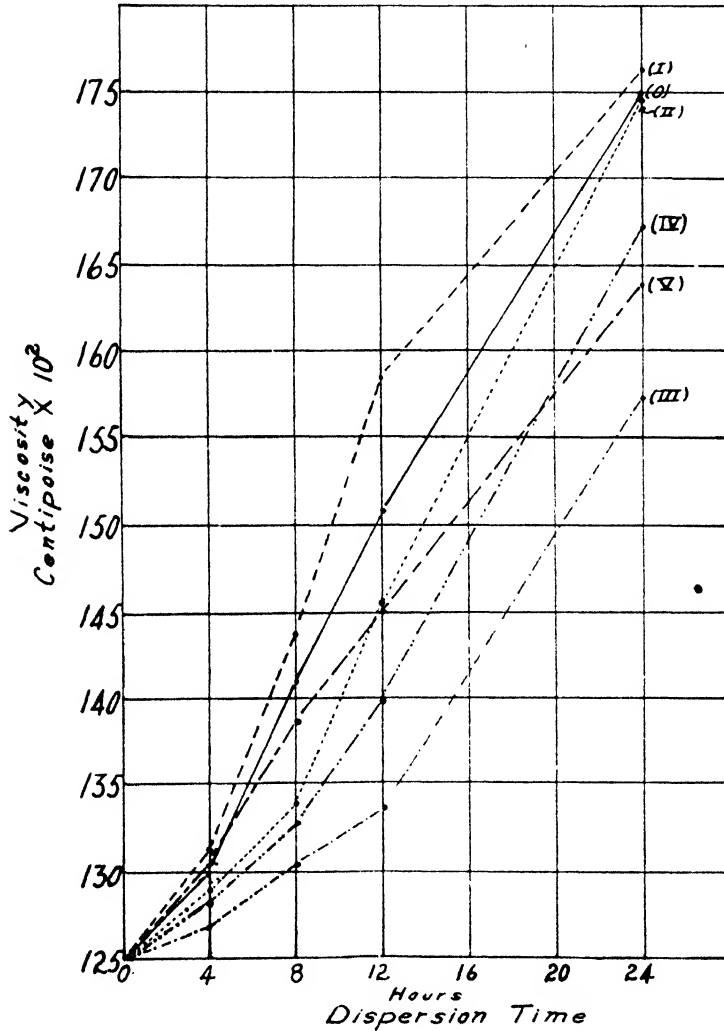


Fig. 2. Relationships between viscosity and time of dispersion of gluten washed from doughs prepared from standard basic formula. Number of curve represents hours of fermentation of dough before gluten was washed.

much more rapidly when treated with papain, and the full effect of the enzyme is apparently felt during the mixing period. There appears to be some evidence in favor of the theory that increasing the fermentation period may have tended to reduce the size of the gluten protein micelle.

The same situation is represented in Figure 5, which shows the relationships when 0.004% papain was added to the dough.

The effect of 0.001%  $\text{KBrO}_3$  upon 0.004% papain in the dough is

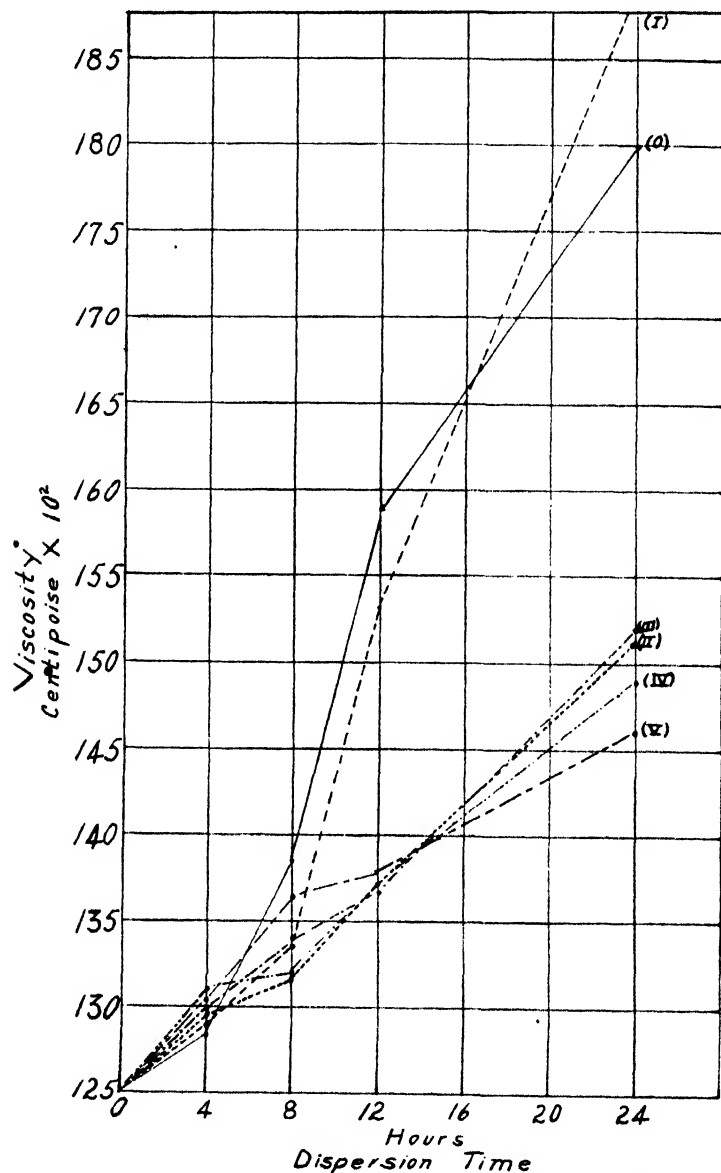


Fig. 3. Relationships between viscosity and time of dispersion of glutes washed from doughs prepared from standard basic formula plus 0.001%  $\text{KBrO}_3$ . Number of curve represents hours of fermentation of dough before gluten was washed.

shown in Figure 6. A gradual decrease in rate of dispersion is evident, this decrease becoming more marked as fermentation time lengthens. No great differences are evident in the unfermented and one-hour-

fermented dough glutens in comparing Figures 5 and 6, thus showing that the bromate did not commence to take effect until the second hour of fermentation was reached. In Figure 7, however, the increase in bromate concentration had a noticeable influence commencing with the

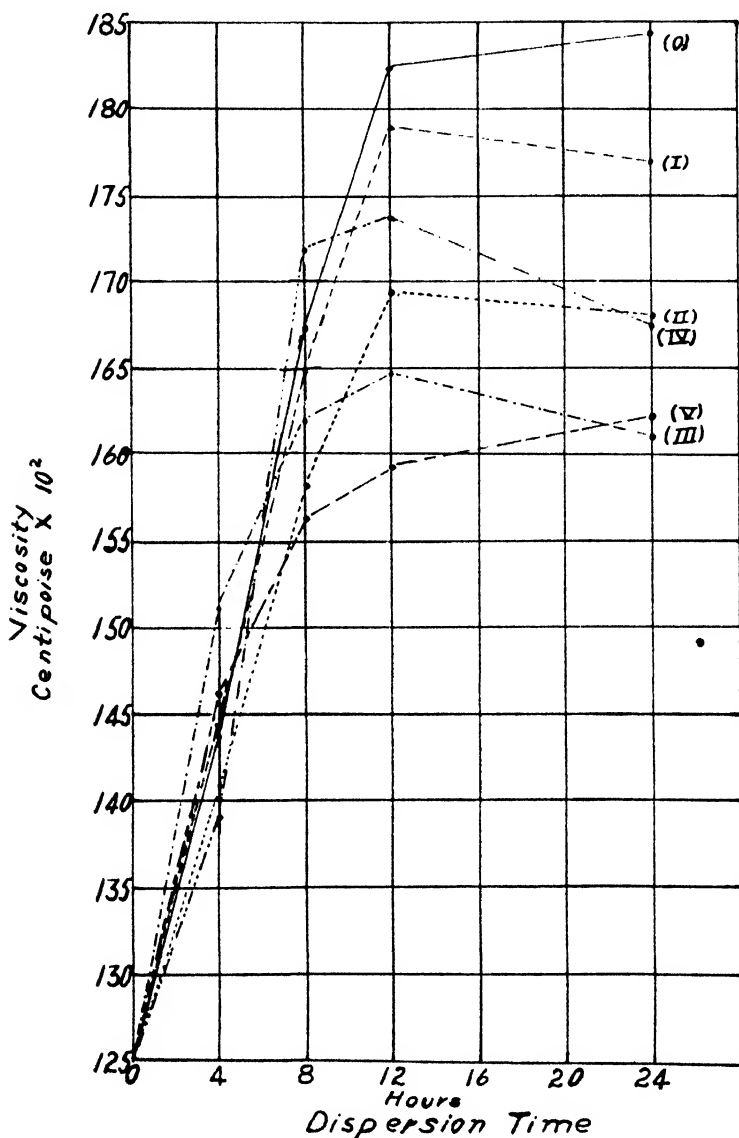


Fig. 4. Relationships between viscosity and time of dispersion of glutens washed from doughs prepared from standard basic formula plus 0.002% papain. Number of curve represents hours of fermentation of dough before gluten was washed.

first hour of fermentation. It is probable that a higher dosage of papain would cause marked differences among the rates of dispersion of the glutens from unfermented doughs, corresponding with results obtained

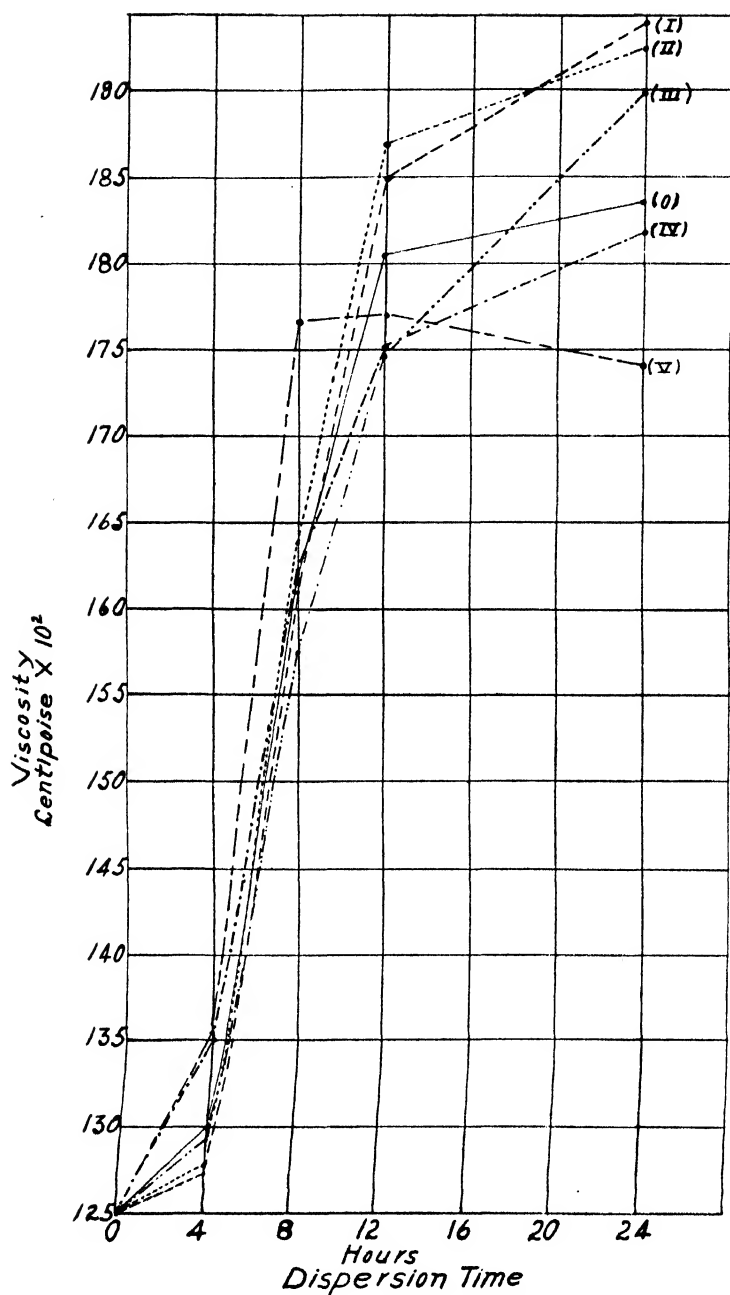


Fig. 5. Relationships between viscosity and time of dispersion of glutens washed from doughs prepared from standard basic formula plus 0.004% papain. Number of curve represents hours of fermentation of dough before gluten was washed.

by the authors in former work of this nature (Harris and Johnson, 1940). It was noticed that the mechanical shaker which was employed in the present study altered the shape of the curves to some extent as

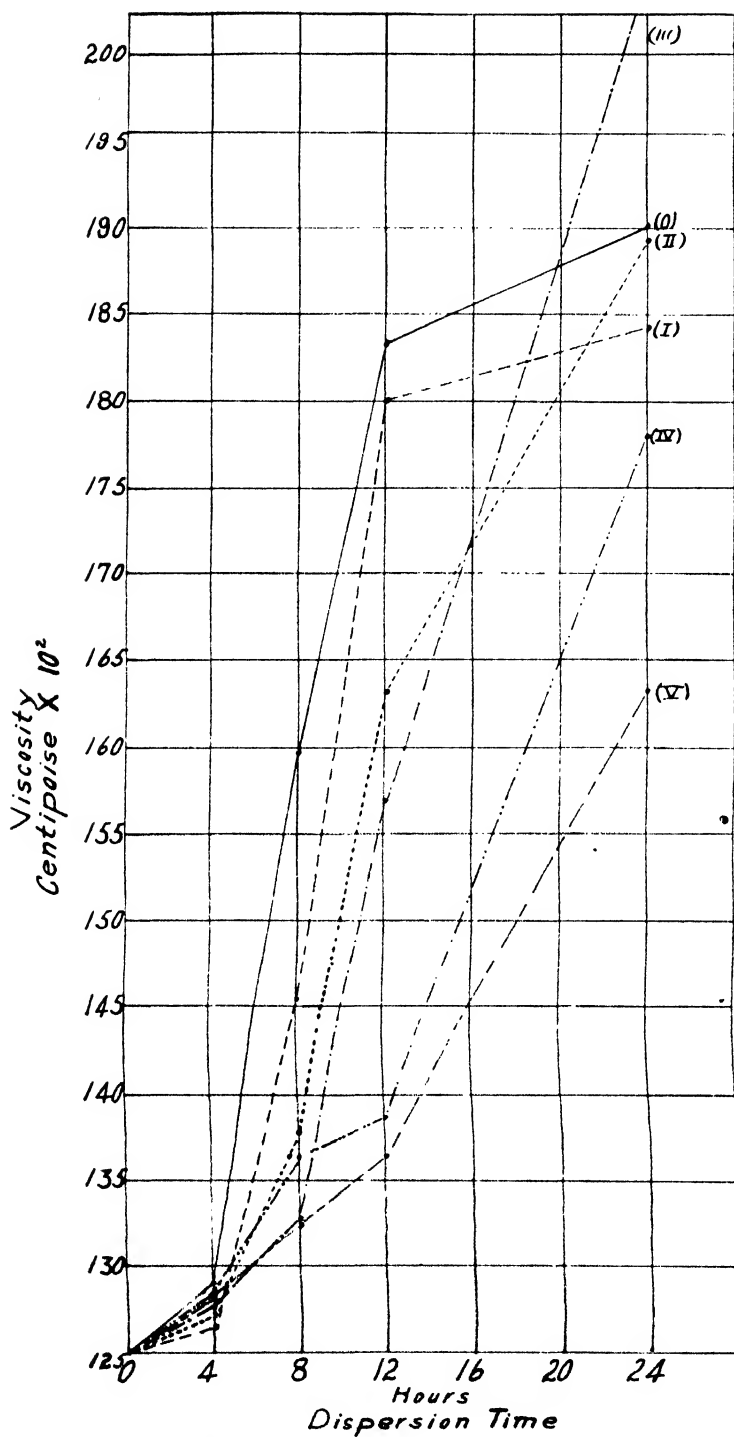


Fig. 6. Relationships between viscosity and time of dispersion of glutes washed from doughs prepared from standard basic formula plus 0.004% papain and 0.001%  $\text{KBrO}_3$ . Number of curve represents hours of fermentation of dough before gluten was washed.



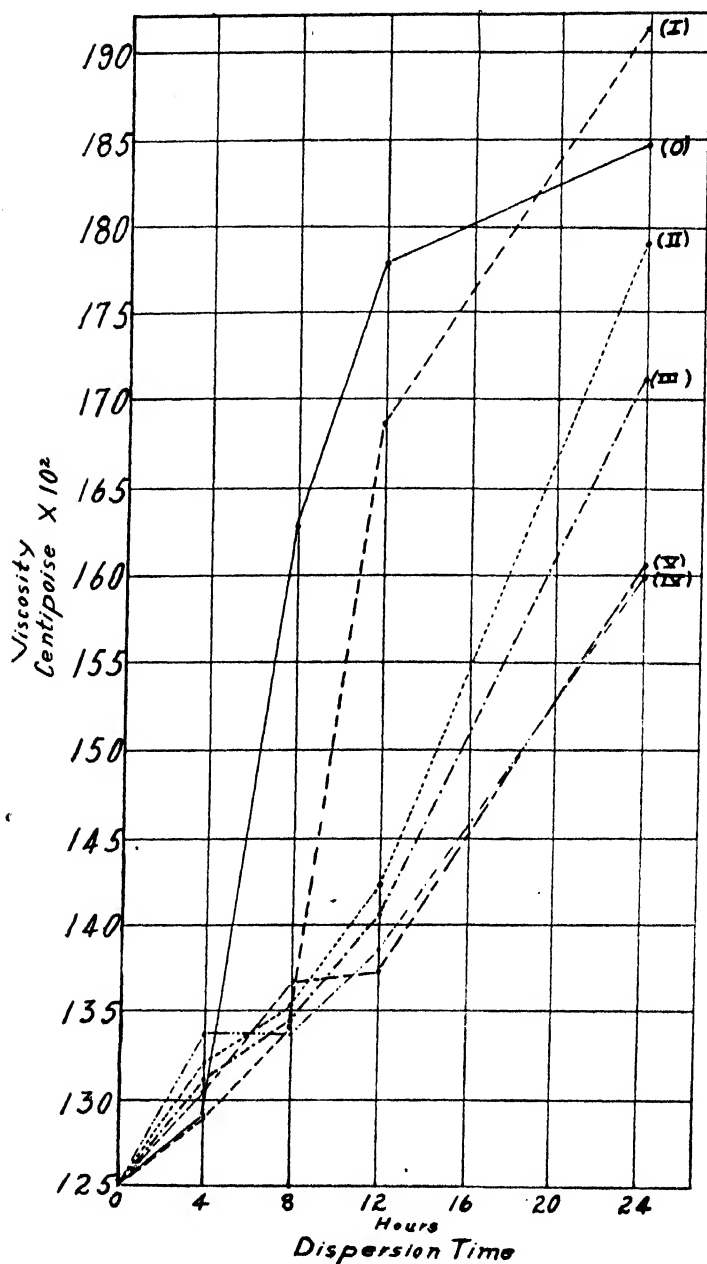


Fig. 7. Relationships between viscosity and time of dispersion of glutens washed from doughs prepared from standard basic formula plus 0.004% papain and 0.004%  $\text{KBrO}_3$ . Number of curve represents hours of fermentation of dough before gluten was washed.

compared to the curves obtained by hand shaking. It is also evident that in the instances of the gluten prepared from the standard basic and bromate-treated doughs which had been fermented for several hours, there was an initial period of "lag" or relatively slow dispersion lasting

from 8 to 12 hours, after which the course of the dispersion was greatly accelerated. This phenomenon could be followed by visual examination of the dispersions. During the first few hours the liquid appeared quite transparent, with the gluten remaining in well defined clumps. After 8 to 12 hours the dispersion began to cloud up and the clumps disinte-

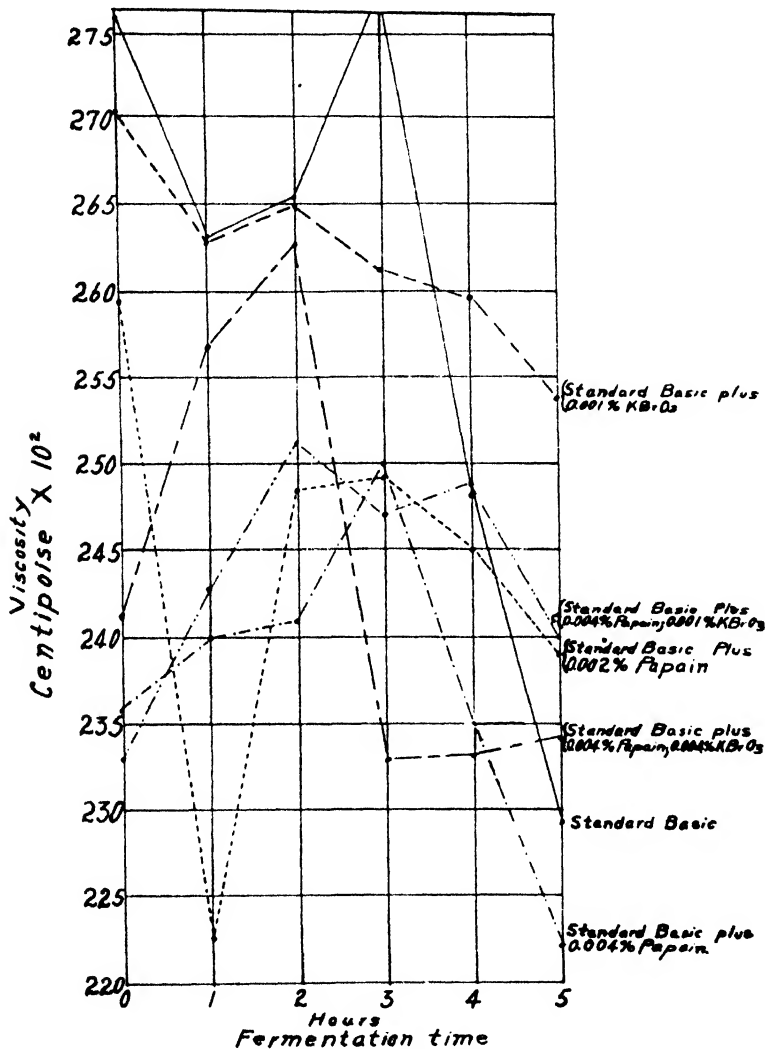


Fig. 8. Relationships between viscosity of gluten dispersion and hours of fermentation for each of the six dough formulas employed.

grated into smaller fragments. In the case of the standard basic-plus-bromate and papain-plus-0.004%-bromate doughs, the effect of the improver was so marked following three or more hours of fermentation that the gluten refused to thoroughly disintegrate after 24 hours of constant shaking.

A further series of dispersions was prepared and continuously agitated on the shaker for 36 hours. They were then centrifuged, and the protein concentration of the liquid was ascertained. The dispersions were next adjusted to a protein concentration of 2500 mg. per 100 cc. and the viscosities determined. In each case the quantity of protein thrown down by the addition of 0.6 cc. of concentrated  $\text{MgSO}_4$  solution

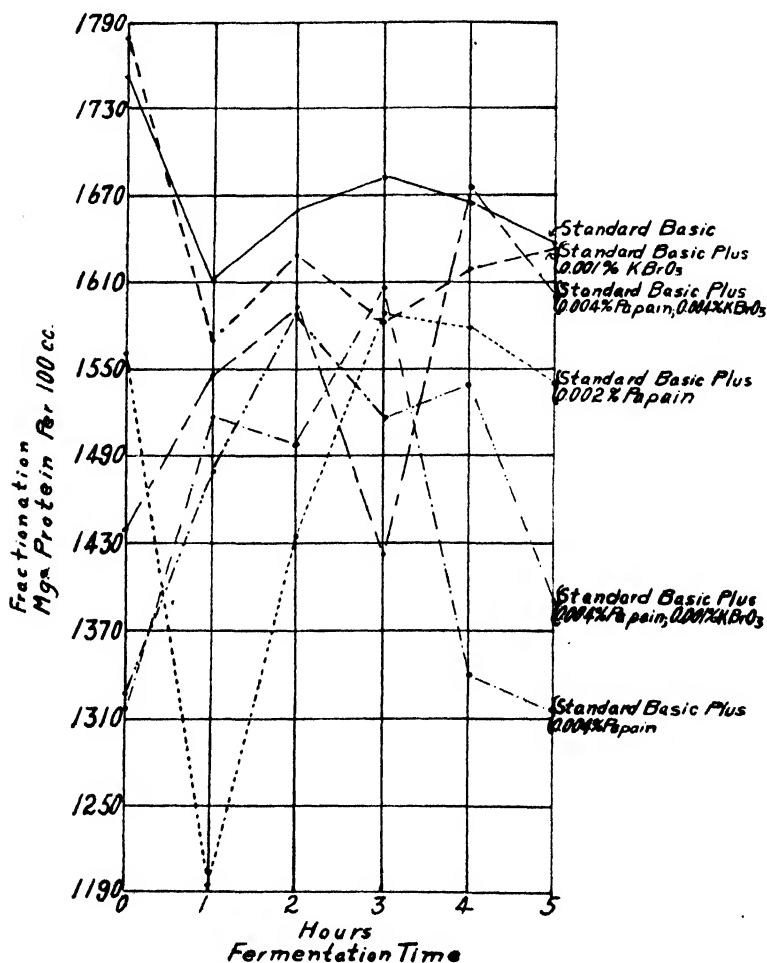


Fig. 9. Relationships between quantity of gluten protein fractionated from dispersion and hours of fermentation time for each of the six dough formulas employed.

to a 10-cc. aliquot of the dispersion was also found. The results obtained, expressed in viscosity and fractionation values for each formula over the fermentation period employed, are shown in Figures 8 and 9, respectively. While a certain amount of variability is evident certain trends can be discerned. From Figure 8 it is evident that in the absence of bromate there is a tendency for viscosity first to decrease and then

to increase to a peak at the second or third hour, followed by a decrease to the end of the fermentation time. In the presence of bromate the initial decrease is modified or disappears entirely and while, in the standard basic doughs, bromate reduces the viscosity peak, it apparently raises this peak in the papain-treated doughs, possibly due to an inhibitory action upon the enzyme. The relationships between fractionation and fermentation time depicted in Figure 9 are roughly similar in nature to the viscosity changes with fermentation period.

Two different effects upon the gluten properties are measured by the studies on the two series of dispersions described. In the first series the influence of the various ingredients and fermentation periods upon the rate of protein dispersion is studied. In the second series the dispersions are on a constant-protein basis, and the effects of formula and fermentation-time variables upon the protein micelle are indicated. These methods of approach to the problem both demonstrate the effect of papain in increasing dispersion rate and reducing effective particle size. Potassium bromate retarded the rate of gluten dispersion and prevented, in some instances, complete dispersion of the gluten. Length of fermentation period apparently increased the effect of bromate upon rate of dispersion, and also affected the size of the protein particle itself.

The changes in pH with fermentation are shown in Table I. A

TABLE I  
COMPARATIVE HYDROGEN-ION CHANGES IN FERMENTING DOUGHS

Dough formula	Hours of fermentation					
	0	1	2	3	4	5
	<i>pH</i>	<i>pH</i>	<i>pH</i>	<i>pH</i>	<i>pH</i>	<i>pH</i>
St. B.	5.85	5.52	5.47	5.37	5.29	5.20
St. B. + 0.001% KBrO <sub>3</sub>	5.85	5.51	5.46	5.37	5.32	5.27
St. B. + 0.002% papain	5.80	5.48	5.46	5.41	5.32	5.28
St. B. + 0.004% papain	5.83	5.49	5.45	5.38	5.34	5.17
St. B. + 0.004% papain with 0.001% KBrO <sub>3</sub>	5.79	5.57	5.45	5.40	5.35	5.22
St. B. + 0.004% papain with 0.004% KBrO <sub>3</sub>	5.79	5.52	5.47	5.35	5.29	5.27

Note—St. B. refers to the standard basic formula with 5% sucrose.

general trend toward a decrease in pH with increase of fermentation time is evident. No differences in pH among the different dough treatments are apparent. These measurements were made directly upon the fermenting doughs by the use of a Beckmann pH meter with the calomel and glass electrodes carefully enclosed in the dough. Each

value shown is the average of five consistent readings. The oxidation-reduction potential values were erratic, and showed no definite trends. Decreasing pH at constant  $E_h$  is commonly thought to denote a tendency toward a decrease in oxidizing power.

A third series of glutens was used for the determination of resistance to pressure with a tenderness tester. In using this apparatus the method of Binnington, Johansson, and Geddes (1939) was followed. The glutens were washed from the doughs and the tests run with minimum loss of time. The gluten was formed into a ball and located under the plunger of the apparatus. Five tests were made on each sample,

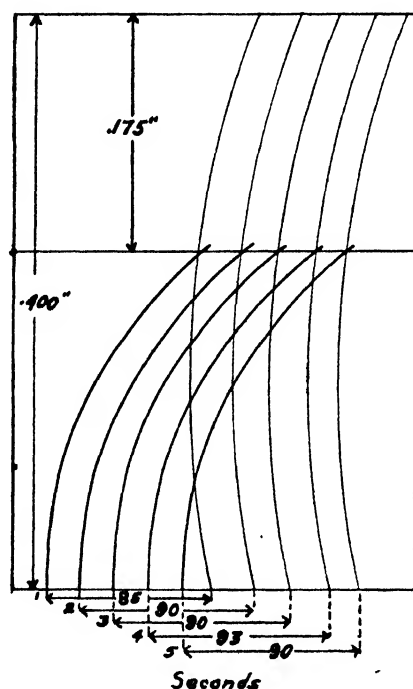


Fig. 10. Representative curves made by five successive determinations of resistance on one sample of gluten. The greater the resistance of the sample the more curved the lines. Figures at bottom represent seconds of time required for kymograph pen to trace the curve, using a uniform mercury flow.

the gluten being dipped under 0.1% sodium phosphate solution (pH 6.8) between tests to avoid drying out. It was found that the final tests on each sample indicated a slight stiffening of the gluten due apparently to manipulation.

A typical record of the resistance test is shown in Figure 10. The values shown are the times taken for the kymograph pen to travel from the start of application of load to the 0.175-inch line, a distance of 0.225 inch. This point was chosen because it occurs after the line has indicated the type of curve and before it has reached the region of ex-

cessive flattening out of the gluten ball where its power of resistance to the deforming force is largely overcome.

The average value for the five determinations was then calculated and used as a single-figure value for a measure of the resistance. Two sets of curves constructed from such average values are shown in Figure 11. It will be noticed that the curves at the left are straighter, *i.e.* rise more rapidly, than the curves at the right, indicating less resistance of the gluten due to the effect of papain as compared with the increased resistance contributed by potassium bromate presumably through inhibitory action upon flour and yeast proteases. Little difference is to

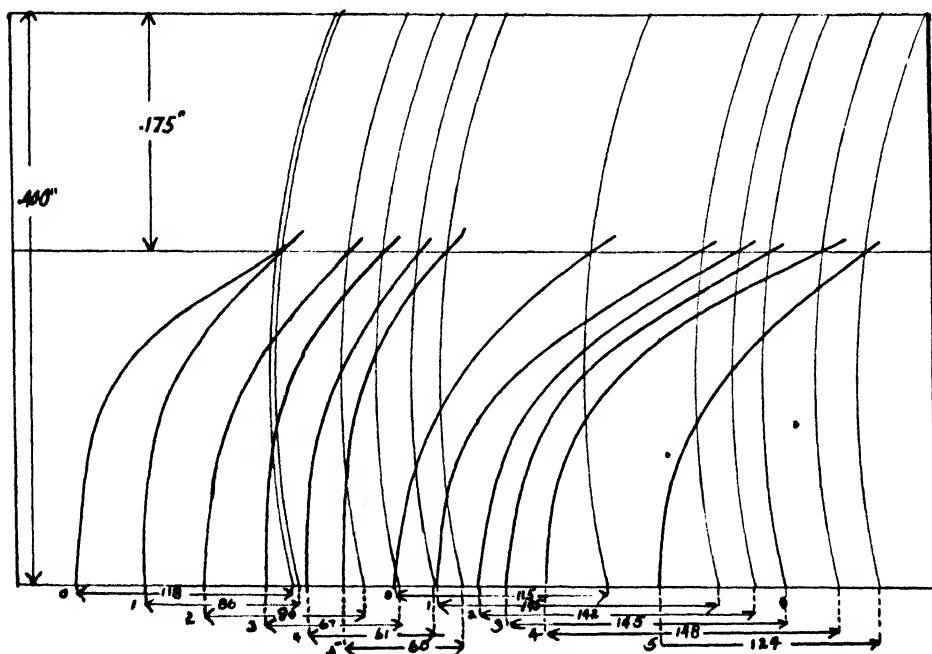


Fig. 11. Resistance curves showing influence of ingredients and fermentation time upon shape of curve. Curves at left represent results obtained with the standard basic formula plus 0.004% papain. Curves at right were obtained with the standard basic formula plus 0.001%  $\text{KBrO}_3$ . Number of the curve refers to hours of fermentation.

be noted between the curves representing different hours of fermentation in the first set of curves, but when  $\text{KBrO}_3$  was added to the basic formula the lines tended to become more curved with length of fermentation period until the fourth hour, indicating that this flour improver definitely stiffens the dough as fermentation progresses. This evidence is in line with baking observations.

Figure 12 represents the changes in resistance with length of fermentation period for the six doughs. Comparing first the two formulas without papain, the stiffening effect of bromate after the first hour of

fermentation becomes apparent. Why the dough without bromate should be initially stiffer than the bromated dough is not clear. Next, in comparing the effects of the two papain increments upon the standard basic formula, it is apparent that the enzyme decreases the resistance of the gluten from the moment the dough is mixed, and that this effect increases with length of fermentation period. This again is in agree-

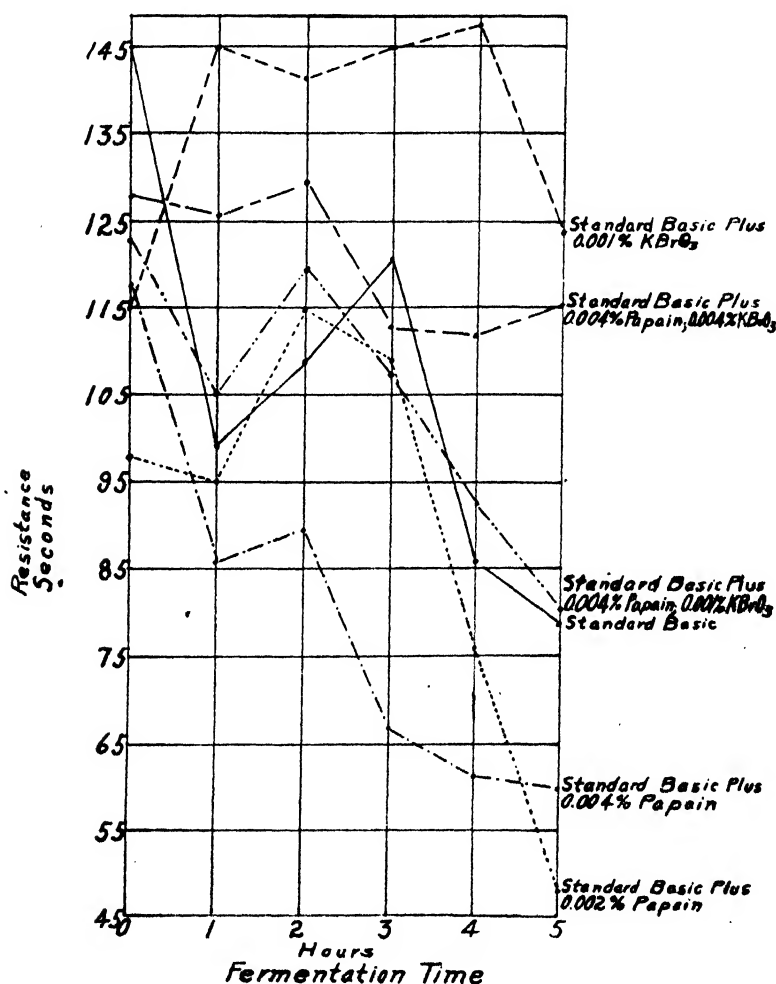


Fig. 12. Change of resistance in seconds of glutes prepared from doughs fermented varying lengths of time.

ment with baking observations which offer evidence of progressive softening of the dough as fermentation progresses in the presence of papain. When bromate is added to the papain-treated doughs a decided stiffening of the gluten is noticeable, especially as fermentation progresses. The data graphically presented in this figure show the same trends in regard to papain and bromate effects as were evident in the viscosity deter-

minations. It is therefore apparent that the physical and biochemical properties of the gluten are affected in comparable manner by either papain or bromate additions.

Correlation coefficients presented in Table II show that a relatively high relationship existed between viscosity and the quantity of protein removed from colloidal solution by  $\text{MgSO}_4$ . The influence of varying fermentation time no doubt lowered this correlation as compared with glutens washed from flour-water doughs.

TABLE II  
CORRELATION COEFFICIENTS COMPUTED FROM THE DATA

Variables correlated		Correlation coefficient $r_{xy}$	$P$
$X$	$Y$		
Viscosity (centipoises $\times 10^3$ )	Protein fractionated (mg.)	+.6960	<.0001
Viscosity (centipoises $\times 10^3$ )	Resistance of gluten (seconds)	+.5547	.0006
Resistance of gluten(seconds)	Protein fractionated (mg.)	+.3043	.0730

### Summary and Conclusions

A series of doughs was prepared from a commercial spring wheat flour using six formulas. These formulas included the standard basic (with 5% sucrose), standard basic plus 0.001%  $\text{KBrO}_3$ , standard basic plus 0.002% and 0.004% papain, and the latter two formulas plus 0.001% and 0.004%  $\text{KBrO}_3$ , respectively.

Three series of glutens were washed by standardized methods from the doughs following fermentation periods ranging from 0 to 6 hours for each formula. Comparative rates of dispersion of the glutens in 10% sodium salicylate were determined on one set, as indicated by changes in viscosity with time. The results showed that glutens prepared from doughs without added papain tended to disintegrate at a lower rate after three hours of fermentation than before, while bromate greatly reduced the rate after two hours of fermentation. The addition of papain without bromate accelerated the dispersion rate and obliterated any effect of fermentation time upon this variable. When  $\text{KBrO}_3$  was superimposed upon papain, however, fermentation behavior reflected the inhibitory action of the oxidizer upon the enzyme.

In a second series, glutens were washed from the doughs, then dispersed, and the protein concentration adjusted to 2500 mg. per 100 cc. of dispersion. The viscosities were determined, and the resulting data showed the effect of papain in reducing this property. There was a marked tendency for the viscosity to increase to a maximum after two



to three hours of fermentation of the dough. Unbromated doughs showed some evidence of an initial decrease in viscosity during the first hour of fermentation. In the presence of bromate this behavior was less apparent. Viscosity decreased after three hours of dough fermentation in every instance. The results obtained by fractionating the protein from the dispersions by suitable additions of  $\text{MgSO}_4$  revealed a somewhat similar picture when due allowance was made for the variability inherent in the biologic material and methods used. Doughs treated with papain showed initial decreases in the quantity of protein removed, corresponding to the viscosity trends. Why such a marked trend should be established only in the unfermented doughs is not clear. Bromate appeared to increase the quantity of protein fractionated in the presence of papain at the beginning and end of the fermentation, but whether it had any effect upon the results obtained at the second or third hour is very doubtful. The 0.004%  $\text{KBrO}_3$  treatment did show a high point at four hours of fermentation, but whether this points to the bromate having a maximum effect at this time cannot be definitely stated.

A third series of glutens was likewise prepared and resistance tests run on glutens molded into small balls using a modification of the technique developed by Binnington, Johansson and Geddes (1939) for measuring the "tenderness" of cooked macaroni.

The data obtained, when plotted against fermentation time, showed a decrease in resistance followed by an increase which reached a maximum at the second or third hour in every dough except one, the standard basic plus 0.001%  $\text{KBrO}_3$ . The gluten from the 0.004% papain treated dough increased little in resistance between the first and second hours, and then decreased sharply. The stiffening effect of  $\text{KBrO}_3$  upon these glutens is quite marked, and tends to increase with the fermentation period. The results obtained with this test are in general agreement with the other determinations employed and illustrate the inhibitive effect of  $\text{KBrO}_3$  upon proteases during dough fermentation. It is not clear from the data whether there is a direct effect of the oxidizing agent upon the gluten properties. The action of papain in increasing the rate of gluten dispersion and in decreasing the viscosity of gluten dispersions, as well as the quantity of gluten fractionated from the dispersions, is clearly evident, as well as its effect in decreasing the resistance or stiffness of the gluten as fermentation proceeds.

Correlation coefficients showed a significant relationship between viscosity and protein fractionated from the dispersions.

## Literature Cited

- Bailey, C. H., and Johnson, A. H.  
1924 Carbon dioxide diffusion ratio of wheat flour doughs as a measure of fermentation period. *Cereal Chem.* 1: 293-304.
- Binnington, D. S., Johannson, H., and Geddes, W. F.  
1939 Quantitative methods for evaluating the quality of macaroni products. *Cereal Chem.* 16: 149-167.
- Bohn, L. J., and Bailey, C. H.  
1936 Elasticity of wheat flour dough. *Cereal Chem.* 13: 389-409.  
1937 Effect of fermentation, certain dough ingredients, and proteases upon the physical properties of flour doughs. *Cereal Chem.* 14: 335-348.
- Brownlee, W. E., and Bailey, C. H.  
1930 Proteolysis in bread doughs. *Cereal Chem.* 7: 487-517.
- Flohl, J. T.  
1936 The effect of chemical flour improvers on proteolytic action in relation to the gas-retaining capacity of fermenting doughs. *Cereal Chem.* 13: 675-689.
- Harris, R. H.  
1938 A study of the effect of proteoclastic enzymes upon wheat gluten solubility in sodium salicylate solutions. *Cereal Chem.* 15: 690-707.  
— and Johnson, John, Jr.  
1940 A study of the effects of proteolytic enzymes and  $KBrO_3$  upon the viscosity and allied properties of dispersions of hard red spring wheat gluten. *Cereal Chem.* 17: 203-222.  
— and Knowles, Darline  
1940 Quality studies on North Dakota durum wheat. *Cereal Chem.* 17: 480-490.
- Henderson, L. J., Fenn, W. O., and Cohn, E. J.  
1919 Influence of electrolytes upon viscosity of dough. *J. Gen. Physiol.* 1: 387-397.
- Jørgensen, Holger  
1935 Über die Natur der Bromatwirkung. *Mühlenlab* 5: 113-126.  
1936 On the existence of powerful but latent proteolytic enzymes in wheat flours. *Cereal Chem.* 13: 346-355.
- Olsen, A. G., and Bailey, C. H.  
1925 A study of the proteases of bread yeast. *Cereal Chem.* 2: 68-86.
- Read, J. W., and Haas, L. W.  
1937 Studies on the baking quality of flour as affected by certain enzyme actions. IV. Potassium bromate and enzyme activity. *Cereal Chem.* 14: 752-766.  
1938 V. Further studies concerning potassium bromate and enzyme activity. *Cereal Chem.* 15: 59-68.  
1939 VI. Further studies relating to the activation and inhibition of flour proteinase. *Cereal Chem.* 16: 60-70.
- Sharp, P. F., and Elmer, R.  
1924 Wheat and flour studies. I. Proteolytic enzymes of flour. I. Auto-digestion of flour milled from frozen and non-frozen wheat harvested at various stages of maturity. *Cereal Chem.* 1: 83-106.  
— and Gortner, R. A.  
1924 The physico-chemical properties of strong and weak flours. VIII. Effect of yeast fermentation on imbibitional properties of glutenin. *Cereal Chem.* 1: 29-37.  
— and Schreiner, O. M.  
1926 Wheat and flour studies. VI. Effect of yeast fermentation on the proteins of flour. *Cereal Chem.* 3: 90-101.
- Smith, E. E.  
1925 Viscosity and baking quality. *Cereal Chem.* 2: 177-190.

# THE INFLUENCE OF THE INITIAL pH OF RYE MASHES ON THE FERMENTATION EFFICIENCY<sup>1</sup>

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(Received for publication April 4, 1940)

The efficiency of rye fermentations (about 83%–86%) had never equaled the efficiency of the corn spirits fermentation (90%–93%) in our plants, and to the best of our knowledge this is the common experience of other plants. The difference in efficiency has been explained in many ways. One possibility is that rye contains a higher percentage of pentosans and other materials which react as starch in an acid-hydrolyzed sample; another, that rye mashes which are not cooked under pressure as is corn, have a greater initial contamination than corn spirits mashes; and there are other possible explanations. Both of the above explanations are plausible. Rye as received has been found to contain a higher percentage of pentosans than corn, and since the maximum temperature of mashing rye is 145° F. (in our procedure), many contaminants capable of development in the fermenter are left in the mash. Most of the other reasons advanced in explanation of the low efficiencies with rye fermentations now appear to have little or no foundation.

Calculations as to the validity of the first explanation for lower efficiency revealed that it could not account for the entire difference. Attention was directed to the fact that the initial pH of a rye mash is about 5.4–6.0, while corn spirits fermentations have a pH of 4.9–5.0 because of the addition of stillage or acid. It was considered probable that this high initial pH of a rye mash permitted large numbers of the contaminants which had survived mashing to grow and produce acid. This acid production would not only be at the expense of the carbohydrate, but moreover might result in the destruction of free amylase activity by lowering the pH of the mash below 4.0 and thus prevent the complete conversion of residual dextrines to maltose.

The results of laboratory tests made by Mr. J. S. Hudson<sup>2</sup> of our department indicated that the yield of alcohol and efficiency of the fermentation were improved by adjusting the initial pH of the mash to 4.8–5.0 with sulphuric acid. This was indirectly corroborated by Kendall (1938) who has recently reported improved alcohol yields during the fermentation of rye mashes by the addition of a small amount of lactic acid, and he assumed that the effect was the result of reduced contami-

<sup>1</sup> This paper was presented at the annual meeting of the Society of American Bacteriologists, New Haven, Conn., December 28, 1939.

<sup>2</sup> Private communication.

nation. However, it was his belief that the cost of the lactic acid would not be recovered in the additional yield of alcohol. Nevertheless, before attempting to introduce pH adjustment into an operating plant it was necessary to conduct experimental fermentations on an intermediate scale. The results of these experiments are reported here.

### Experimental

A series of experiments was conducted at the Lawrenceburg, Indiana, plant and the mashes used in the experiments were portions of the regular mashes prepared at that plant. The rye mashing procedure used is as follows: The water is drawn into the pony mash tank at a temperature of 110° F., the malt is added while the water is agitated, and the slurry is then added to the cooker which contains water at a temperature of 100° F.; after all the malt is added, finely ground rye is introduced slowly while the mash is constantly agitated. The mash is mixed for 10 minutes at this temperature. The temperature is then raised to 130° F. in 30 minutes and held for 45 minutes, thus allowing some proteolysis and some conversion of starch, which results in a thinner mash. The temperature is then raised to 145° F. in 15 minutes and held for one hour; at this time the mash is pumped through the coolers and into the fermenters. Four cooks and the yeast mash fill the fermenter. The final volume and setting temperature are adjusted with stillage and water. The yeast is usually added to the fermenter with the first cook. This addition is made about 4 to 6 hours before the fermenter is completely filled with the other three cooks and the stillage or water.

For the experimental work, as soon as all of the rye mash was in the fermenter and usually before any stillage or water was added, 50 gallons of mash was removed from the fermenter, thoroughly mixed, and split into two 25-gallon portions which were placed in 55-gallon drums that had just been steamed for one hour. The drums were then placed next to the parent plant fermenter. By the addition of 6 normal sulphuric acid the pH of one of the two small fermenters was lowered to a pH between 4.8 and 5.0. To the second fermenter an equal volume of distilled water was added to correct for the dilution of the other mash and this served as the unadjusted control. Both fermenters were then covered with the lids of the drums. In this manner such variables as differences in grains, mashing conditions and concentrations, yeast used for inoculum, and the incubation temperature were eliminated. The pH, Balling, and titratable acidities were determined, and every 24 hours the same determinations were repeated. Occasionally it was necessary to omit the usual three-hour pH and temperature determinations. The fermenters were sampled for final data at the time

the parent plant fermenter was pumped to the beer well for distillation. This was after approximately 72 hours. The final data consisted of duplicate alcohol determinations, pH, Balling, titratable acidity, and residual sugar determinations. The alcohol content was determined by distillation of a known volume of mash, followed by the analysis of the distillate with a Zeiss refractometer. The residual sugars were determined on samples of supernatant liquid from centrifuged samples. Each sample was acid hydrolyzed and the sugar content determined by the method of Stiles, Peterson, and Fred (1926).

Table I lists the results of eight experiments. The variations in the final alcohol content among experiments are due to variations in the

TABLE I  
A SUMMARY OF THE PERTINENT DATA ON 25-GALLON EXPERIMENTAL RYE  
FERMENTATIONS WITH THE pH ADJUSTED AND ON  
THE CONTROL FERMENTATIONS

Experi- ment	Fermenter <sup>1</sup>	Initial pH	Final data			
			pH	Titratable acidity  % acetic	Residual sugar  g./100 cc.	Alcohol content  % by vol.
1	a	5.50	3.75	0.68	0.96	5.17
	b	5.11	3.76	0.63	0.96	5.30
2	a	5.38	3.75	0.66	1.05	5.37
	b	4.85	3.75	0.62	1.00	5.53
3	a	5.45	3.72	0.71	1.14	5.83
	b	4.88	3.73	0.67	1.06	5.99
4	a	5.45	3.72	0.68	1.10	5.24
	b	4.88	3.78	0.64	1.04	5.37
5	a	5.58	3.70	0.69	1.31	5.20
	b	4.70	3.82	0.59	1.02	5.63
6	a	5.50	3.58	0.87	1.39	5.41
	b	5.10	3.73	0.74	0.92	5.52
7	a	5.37	3.65	0.87	1.15	5.12
	b	5.00	3.81	0.68	1.02	5.52
8	a	5.72	3.68	0.82	0.96	5.43
	b	4.98	3.75	0.73	0.84	5.63

<sup>1</sup> Fermenter *a* served as the control (pH unadjusted), the pH of fermenter *b* was adjusted to 4.8–5.0 in all cases at the beginning of the experiment.

concentrations of the mash used. This was the result of sampling the plant fermenters after varying amounts of stillage or water had been added to them. Figure 1 is a representative graph showing the pH curves for the experimental fermenters and for the plant fermenter, re-

spectively. Toward the end of the experiment it was impossible to obtain data every three hours; hence there are fewer points on the graph for the final stages of the fermentation. As may be noted from Table I the alcohol content was higher in every case in those fermentations with the pH adjusted to 4.8–5.0. This average increase is 0.22% by volume of alcohol. Thus the efficiency of the fermentation was raised by adjustment of the initial pH to 4.8–5.0. The variations between sets of fermentations are due to the fact that it was impossible to sample the plant fermenters when the same dilution of the mash with stillage had occurred each time and thus the initial sugar content varies

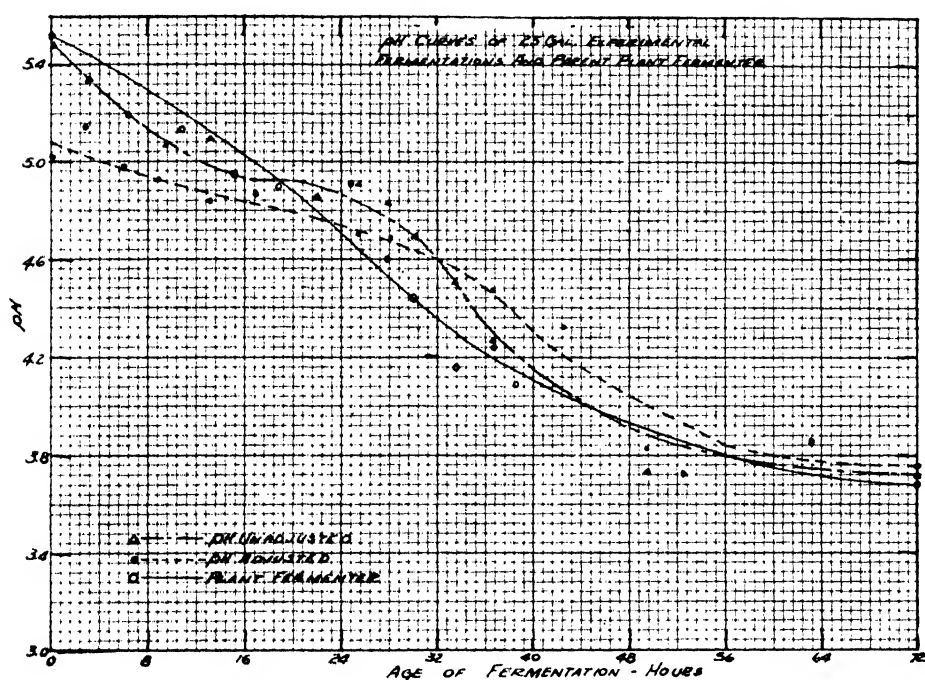


Fig. 1.

from experiment to experiment. Table I also shows that with the exception of Experiment 1, less residual sugar is present in those fermentations in which the initial pH was adjusted.

One of the best indications of the degree or extent of contamination is the titratable acidity in the fermentation; a high acidity indicates greater contamination. As may be seen, the final titratable acidity is higher in all experiments when the initial pH of the fermentation is unadjusted; thus it would appear that initial pH adjustment prevents contamination and acid production.

As a result of the evidence presented in Table I it was decided to adopt pH adjustment of rye fermentation mashes in plant practices.

This was done, and by the addition of from 5 to 8 gallons of 66° Be. sulphuric acid to 40,000 gallons of mash the initial pH was lowered to 4.8–5.0. The results of eight plant fermentations, using this procedure, are presented in Table II. The efficiency (91.5%) was calculated from the starch analysis of the grain and the actual yield of alcohol from plant distillation. This efficiency with rye fermentation is the highest, by 5%–6%, ever obtained in our plants or in others, to the best of our knowledge, and has been successfully maintained since the introduction of initial pH adjustment.

TABLE II

EIGHT RYE MASH PLANT FERMENTATIONS IN WHICH THE INITIAL pH WAS ADJUSTED  
Mash Bill—80% Rye, 2% Barley Malt and 18% Rye Malt

Experiment	Initial pH	Alcohol yield (proof gallons/bushel of grain)	
		Analytical	Actual
1	4.96	4.76	4.68
	5.03 distilled		
	4.83 together		
	5.04		
2	4.87 distilled	4.69	4.71
	5.04 together		
3	4.97 distilled	4.62	4.64
	4.93 together		

Average fermentation efficiency = 91.5% of theoretical calculated from the actual plant yield.

### Discussion of the Data

As previously stated it was believed that the degree of bacterial contamination with resultant acid production and loss of free amylase activity was largely responsible for the low alcohol yields in rye fermentation. The data presented in this paper support this theory. In Figure 1 it will be seen that the pH curves for the unadjusted fermentation and the plant fermentation are very nearly identical. The pH remains above 5.0 for the first 12 to 16 hours during which time more contaminants may develop than in the fermentation with the pH adjusted. After 20 to 24 hours the pH for the unadjusted fermentation falls below the level of the adjusted fermentation and then falls below 4.0 at the end of 40 hours, which is about 4 hours earlier than for the fermentation with the initial pH adjusted. Below a pH of 4.0 free amylase activity disappears. Occasionally the pH of the adjusted fermentation will fall below 4.0 at about the same time as the unadjusted fermentation; this is, of course, affected to some extent by other factors, *i.e.* initial contamination, mashing conditions, etc. None the less, a fermentation with the

initial pH unadjusted is subjected to more opportunity for the growth of acid-producing contaminants (which apparently develops) than one in which the initial pH is lowered to 4.8–5.0, and the unadjusted mash is ordinarily in a pH range favorable to amylase activity for a comparatively shorter period of time.

The foregoing postulations are well supported by the data in Table I. The lower final titratable acidity values in the eight fermentations indicate less contamination with acid-producing bacteria and the lower residual sugar content in the eight groups of fermentations shows that amylase activity has been greater, with the result that more dextrine was converted to maltose which was subsequently fermented.

### Summary

It has been shown that by adjusting the initial pH of rye mashes to 4.8–5.0 through the addition of sulphuric acid to the mash the efficiency of the fermentation was increased by approximately 4% in a series of test fermentations. This increase in efficiency appears to be due to a reduction in the growth of contaminants, with comparatively less loss of carbohydrate, with less acid production, and presumably with a greater protection to the amylase activity.

When this technique was placed in plant practice the efficiency of plant-scale rye fermentations was raised from 83%–86% to 90%–92%.

### Acknowledgment

The authors gratefully acknowledge the assistance of Mr. J. S. Hudson, Mr. R. E. Scaff, Mr. Frank Gallagher and Mr. G. A. Ratti of the Seagram Research Department in conducting this series of experiments.

### Literature Cited

Kendall, A. R.

1938 The fermentation of rye. *Proc. S. D. Acad. Sci.* **18**: 26–36.

Stiles, H. R., Peterson, W. H., and Fred, E. B.

1926 A rapid method for the determination of sugar in bacterial cultures. *J. Bact.* **12**: 428–435.



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